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THE HAEMOGLOBIN AND SOLIDS OF THE BLOOD OF AUSTRALIAN ABORIGINES AND WHITES

by

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(Submitted for publication 10th November, 1934.)

Several investigators have shown that conditions which lead to an increased loss of water from the body by evaporation cause alterations in the proportion of water and solids in the blood. Haldane and Priestley (1916) showed an increase in the haemoglobin content of the blood of human subjects after profuse sweating, although Hunt (1912) had failed to observe any change. Young, Breinl, Harris, and Osborne (1920) observed an increase of blood solids and of refractive index of the serum under similar conditions. Barcroft, et al. (1923) observed that the blood volume increased with rising environmental temperature. Barbour and his colleagues, in a long series of investigations on man and animals (Barbour, 1923; Barbour and Tolstoi, 1923; Barbour, Loomis, Frankman, and Warner, 1924; Hamilton, Barbour, and Loomis, 1924), have shown that the essential response of the blood to conditions of increasing difficulty of heat loss is a dilution followed by a concentration.

Rogers and Lackey (1928) have confirmed the work of Barbour and Hamilton (1925) and of Hamilton and Barbour (1925) which showed by experiments on dogs with excised and denervated spleens that the response of their blood solids to variations of temperature and humidity is not due to variation in the number of circulating erythrocytes.

The work of McIntosh, Kajdi, and Meeker (1930), and of Barbour and Gilman (1934) indicates that the changes of blood concentration are due primarily to changes of body temperature rather than to changes in the water content of the body.

The striking differences between the evaporative loss from whites and aborigines under summer conditions in Central Australia, and whites under milder conditions in Sydney, which have been shown by Wardlaw, Davies, and Joseph (1934), suggest that corresponding variations of the solid content of the blood of these subjects are to be expected.

The present investigation was carried out during January and February (1933 and 1934) in the vicinity of Hermannsburg and Mt. Liebig, and during October and November in Sydney.

The total solid content of the blood was measured by drying 0·1 ml. of blood from a calibrated pipette to constant weight in a water oven. Haemoglobin was determined by the method of Newcomer (1919). The glass standard was checked by determination of oxygen capacity with the Van Slyke manometric apparatus. Results are recorded as gm. per 100 ml. of blood. It was not always possible to obtain simultaneous specimens for the two estimations.

The subjects examined were healthy adult males. Nearly all of the white subjects were engaged in academic work. All of the aboriginal subjects were full-bloods. Three groups of aborigines were examined: Aranda living habitually under mission conditions at Hermannsburg; wild Aluritja from the vicinity of Mt. Liebig living under mission conditions during the period of examination; wild Aluritja living under their natural conditions. Only one subject was over 45 years of age.

RESULTS.

The values obtained for twenty-six Australian whites under Sydney conditions are shown in Table 1.

TABLE 1.

Total Blood Solids and Haemoglobin (gm./100 ml.) of Male Whites in Sydney. Mean Maximum Temperature 22·7°C., Mean Diurnal Temperature 18–5°C., Mean Relative Humidity 63 p.c., Mean Pressure of Water Vapour, 9·8 mm. Hg., Height above Sea Level 138 ft.

Subject.	Total Solids.	Haemoglobin.	Subject.	Total Solids.	Haemoglobin.
Bar	22·9	15·1	Eas	24·4	15·1
Jos	23·0	15·7	Dat	24·0	14·6
War	22·8	14·4	Edw	23·4	15·1
Dav	21·8	15·0	Plo	25·0	16·0
Jon	25·1	15·7	Mel	22·4	15·4
Dee	23·8	15·6	Bri	24·7	16·1
Tho	24·2	15·3	Hal	21·6	16·1
Ueb	22·1	16·4	Rya	24·5	15·0
Lev	26·3	16·5	Cra	22·8	17·8
Bon	24·2	16·7	Ell	24·0	18·6
Bro	21·5	13·7	Coo	24·6	15·9
Ash	24·6	16·6	Dav	22·9	15·5
Moo	23·3	16·6	Rob	22·3	17·1
			Mean	*23·55*	*15·83*

The mean value for the haemoglobin in this group is very close to that found for males by Jenkins and Don (1933) in Britain, by Wintrobe (1933) in North America, and to the figures collected by Price-Jones (1931) for comparable determinations in various parts of the world.

In Table 2 the figures are given for a group of thirteen whites examined in Central Australia.

TABLE 2.

Total Blood Solids and Haemoglobin (gm./100 ml.) of Whites in Central Australia. Mean Maximum Temperature 36°C., Mean Diurnal Temperature 28°C., Mean Relative Humidity 35 p.c., Mean Pressure of Water Vapour 9·8 mm. Hg., Height above Sea Level about 1,600 ft.

Subject.	Total Solids.	Haemoglobin.	Subject.	Total Solids.	Haemoglobin.
Bar	22·0	14·0	Jos	20·8	14·5
Cou[1]	22·7	16·0	Lac	21·9	14·4
Dav	20·2	14·6	McD	21·6	15·2
Dav[2]	21·2	14·8	Mil	23·9	16·3
Fre	21·2	14·5	Rob	21·6	16·2
Jef	22·1	16·2	War	22·4	13·6
Joh[1]	21·1	14·0	*Mean*	*21·67*	*14·95*

[1] Permanent resident. [2] One year later.

The mean values of the results in this Table are distinctly lower than the figures for the Sydney whites.

In Table 3 the figures are given for a group of five whites who were examined both in Sydney and in Central Australia.

TABLE 3.

Total Blood Solids and Haemoglobin (gm./100 ml.) of Same Whites in Sydney and in Central Australia.

	Sydney.		Central Australia.	
Subject.	Total Solids.	Haemoglobin.	Total Solids.	Haemoglobin.
Bar	22·9	15·1	22·0	14·0
Dav	21·8	15·0	20·2	14·6
Jos	23·0	15·7	20·3	14·5
Rob	22·3	17·0	21·6	16·2
War	22·8	14·4	22·4	13·6
Mean	*22·16*	*15·46*	*21·30*	*14·56*

The mean figures for the two environmental conditions shown in Table 3 differ in the same direction and to about the same extent as the mean figures for the larger groups of whites.

Table 4 gives the figures for two groups of aborigines examined under mission conditions: nine Aluritja and sixteen Aranda.

TABLE 4.

Total Blood Solids and Haemoglobin (gm./100 ml.) of Central Australian Aborigines. Mean Maximum Temperature 36°C., Mean Diurnal Temperature 28°C., Mean Relative Humidity 35 p.c., Mean Pressure of Water Vapour 9·8 mm. Hg., Height above Sea Level about 1,600 ft.

Aluritja Subject.	Total Solids.	Haemoglobin.	Aranda Subject.	Total Solids.	Haemoglobin.
Imb	21·9	14·4	Alb	23·0	15·1
Nur	23·3	14·3	Fre	22·3	15·5
Wur	22·2	16·1	Lau	23·9	18·2
Kat*	22·1	12·5	Joe	21·0	15·3
Ind	22·8	14·9	Gus	21·5	15·1
Pam	23·2	15·1	Jer	22·1	13·7
Nan	23·4	15·8	Ale	22·2	14·1
Huk	22·7	14·7	Kus	22·2	15·3
Nar	24·0	15·0	Eno	22·4	14·9
Mean	*22·84*	*14·76*	Har	23·6	15·1
			San	23·0	16·4
			War	24·7	16·8
			Pet	24·8	14·9
			Ter	23·0	14·6
			Men	23·2	15·0
			Sim	22·7	15·0
			Mean	*22·85*	*15·31*

* Subject about 65 years old.

The mean figures for the two groups in Table 4 lie very close together. The small difference between the haemoglobin values, as will be shown, is without significance.

The results of determinations of total blood solids only, made on a group of eighteen Aluritja examined in the field, are shown in Table 5.

TABLE 5.

Total Blood Solids (gm./100 ml.) of Aluritja living under Natural Conditions. Temperature, Humidity, and Height as in Table 2.

Subject.	Total Solids.	Subject.	Total Solids.
Tji	22·4	Jup	21·0
Uji	21·3	Nga	21·0
Wan	22·1	Nan	22·3
Bei	21·5	Kat	21·2
Nab	21·1	Kur	21·4
Wang	20·2	Tap	20·9
Imu	22·0	Tal	22·3
Wil	21·2	Win	20·6
Mur	21·5	Kata	21·8
		Mean	*21·4*

The mean value of the results in Table 5 is close to that of the whites in Central Australia, but distinctly lower than that for aborigines examined under mission conditions.

In Table 6 the effect of exercise on the total blood solids of white and black

subjects is shown. The blacks were members of the group of Aluritja examined under their natural conditions. The first white subject in the table and the two black subjects climbed to the top of a hill about 500 ft. high, and back. The second white subject walked a distance of four to five miles. The experiments were carried out between 3·9 and 4·30 p.m. The exercise produced copious sweating in all of the subjects.

TABLE 6.

Effect of Exercise at High Temperature and Low Humidity (see Table 2) on Total Blood Solids (gm./100 ml.) of Whites and Aborigines.

Subject.	Total Blood Solids. Before exercise.	After exercise.	Rise.
Dav (white)	21·5	23·5	2·0
Jef (white)	22·1	25·0	2·9
Tji (black)	22·3	22·4	0·1
Wan (black)	22·1	22·6	0·4

The figures in Table 6 show that the white subjects differed sharply from the blacks in the response of their blood solids to exercise under summer conditions in Central Australia. The whites showed a distinct increase in the concentration of their blood solids. The blacks showed a very slight increase.

DISCUSSION.

Although distinct differences appear between the mean values of the results for certain groups of subjects, these differences cannot be assumed to be significant without further examination. No extreme variations occur among the individual results, but even under these conditions mean figures are sometimes deceptive. The results have been examined by the method described by Dunn (1929). For the series to be compared, the distributions of the individual results about some convenient arbitrary value were ascertained and expressed as fractions of the total number results above and below the arbitrary value. The standard deviation of the difference between the deviations was then calculated.

Examination of the results in this way confirms the impression given by the means. In no case in which the difference between two means is more than 5 p.c. of the lower figure does the standard deviation exceed 0·36 of the difference. This ratio corresponds to less than two chances in 1,000 that the difference is not significant. In most cases the chance against the significance of the difference is smaller.

The figures for the haemoglobin content of the blood of the Sydney whites show that these subjects do not differ significantly from comparable subjects in other countries in this respect. The mean figures for the whites in Central Australia, however, are significantly lower. The mean value of the haemoglobin in

the blood of the aborigines is also significantly lower than that of the Sydney whites. It does not differ significantly from the figure for the whites in Central Australia.

The mean concentration of total blood solids is also significantly lower for the whites in Central Australia than for those in Sydney. This figure is also significantly lower than that for aborigines under mission conditions. The figure for these aborigines is slightly, but not significantly, lower than that for the Sydney whites. On the other hand, the mean figure for the aborigines in their natural state is significantly lower than that for the aborigines under mission conditions and that for the Sydney whites. It does not differ significantly from that for the whites in Central Australia.

These results indicate that the whites in Central Australia had entered the dilution phase of the response of their blood to increased environmental temperature. Although the average relative humidity in this region was considerably lower than that in Sydney, the average pressure of water vapour was almost the same in both regions (9·8 mm. Hg.).

The blood of the aborigines living under mission conditions appears not to have entered the dilution phase. Under the more strenuous conditions of their natural life, however, they had definitely entered this phase.

The figures for the total blood solids of whites after exercise show that these subjects had then entered the second or concentration phase of the response of their blood to increasing difficulty of heat loss. The aborigines did not reach this stage in spite of the freer use which they make of water for cooling purposes.

These, and the results obtained under less strenuous conditions, both point to the aborigines being able to adapt themselves to the conditions examined with less modification in the composition of their blood than was necessary to the whites.

SUMMARY.

The mean haemoglobin content of the blood of 26 healthy adult male whites in Sydney was 15·83 gm./100 ml.; for 13 similar whites in Central Australia it was 14·95 gm./100 ml. Examination of the same whites in both regions showed that difference of environment was responsible for the difference in haemoglobin. The mean figure for 25 comparable full-blood aborigines was 15·11 gm./100 ml.

The mean total solid content of the blood of the whites in Sydney was 23·55 gm./100 ml., and in Central Australia, 21·67 gm./100 ml. The mean figure for the above aborigines, examined under mission conditions, was 22·85 gm./100 ml. For 18 aborigines in the natural surroundings it was 21·43 gm./100 ml.

The increase of blood solids resulting from exercise was much greater in the whites than in the blacks examined.

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THE INFLUENCE OF HEAVY WATER ON PLANTS

by

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Various investigations have been published on the influence of heavy water on plants and animals, but many of these investigations appear to have been hurriedly conducted and in some cases without adequate regard to the precautions necessary in carrying out experiments with culture solutions, such as maintenance of standard pH in culture solutions and controls, the effect of solubility of culture salts in ordinary and heavy water solutions, and the influence of the distillation apparatus on the ordinary and heavy water samples. Some preliminary investigations carried out in this laboratory showed that the influence of the mode of distillation was quite noticeable and capable of causing diverging results according to the material used. In addition it was found that sterilization alters the pH value of Clark's solution in both heavy water (1 : 1,000) and in distilled water. Before sterilization Clark's solution had the value of pH 6·07 in both heavy water and in distilled water; after sterilization the pH in the heavy water solution was 5·55 and in distilled water 5·15.

To avoid these errors and the disturbing effect of adding buffer salts or acid to standardize the pH value or to diminish its effect, the original samples of heavy water and ordinary distilled water were distilled using the same glass still throughout. In this way it was found possible to obtain culture solutions containing heavy water, and containing none, with practically identical pH values, namely 6·60 and 6·63. Several discrepancies in the original results obtained were then cleared up.

Many general references to the physiological effects of heavy water have been made. From these it seems clear that the influence of heavy water varies according to its concentration and also to the organism used, and that it is not possible at present to lay down any definite laws for its action. There seems, however, to be a general consensus of opinion that in diluted form it favours the growth of *Spirogyra*.

Lewis (1933) found that tobacco seeds would not germinate in pure heavy water, and that in a 50 p.c. solution they germinated less readily than in pure water. Lewis (1934) also gives a fairly full account of the biology of heavy water.

Barnes (1933) found that *Spirogyra* lived longer in heavy water of specific gravity 1·000061 than in distilled water; the filaments shewed less movement, and had a tendency to fragmenta-

tion. The chlorophyll containing organism *Euglena*, in a constant concentration of heavy water, increased in numbers and activity compared with cultures in ordinary water (Barnes, 1934). Meyer (1934) found that heavy water accelerates the growth of *Aspergillus*, and Richards (1933) records similar results with yeast.

According to Washburn and Smith (1934), a growing willow tree exercises a preferential selection of the heavy hydrogen isotope in the process of synthesizing organic compounds, and thus lessens the percentage of heavy water in the surrounding solution, but Stewart and Halcombe (1933) found that the concentration of heavy water in the urine and milk of animals was the same as in the water they absorbed.

It seems probable that pure heavy water would be lethal to all ordinary organisms not previously adapted to it because of the possible disturbance which a transference from ordinary to heavy water causes in certain vital functions. In varying degrees such transference will affect surface tension, imbibition, osmotic pressure, solubility of food materials, photosynthesis and the action or production of enzymes. There is, however, little physiological value in such extreme experiments. Far more information is to be obtained by using dilute concentrations of heavy water in which the organisms can live indefinitely, and observing which functions are affected at various concentrations. Accordingly, as fairly large supplies of heavy water in a concentration of 1 per 1,000 were made available by Professor Laby, tests were carried out on a number of chlorophyllous and non-chlorophyllous organisms by members of the Botany School.

CHLOROPHYLLOUS PLANTS.

Lemna minor. Cultures were made in Clarke's physiologically balanced solution, $CaH_4P_2O_8$, 0·094 gm.; KNO_3, 0·808 gm.; $MgSO_4$, 0·12 gm.; $FeCl_3$, 0·01 gm. in 1,000 c.c. The flasks were sterilized, and to each an equal number (2 or 3) of fronds of *Lemna* added, choosing fronds of equal size. The experiments were done in triplicate, and the fronds counted after exposure to uniform illumination on a rotating stand for three weeks. The results are given in the following table:

	Number of Fronds.			
Experimental procedure.	A.	B.	C.	Average.
A. In original heavy water (1: 1,000) sterilized; pH 5·55	19	12	15	15
In distilled water sterilized; pH 5·15	21	16	21	19
B. In glass distilled heavy water (1: 1,000) sterilized; pH 5·2	38	44	38	40
In glass distilled water sterilized; pH 5·0	30	28	35	31
C. Heavy water as in B, but unsterilized; pH 6·6	—	—	—	74
Distilled water as in B, but unsterilized; pH 6·6	—	—	—	66

In the first experiment the heavy water slightly decreased the rate of growth, but as it was thought that the difference in pH value caused by sterilization might produce this effect the waters were distilled through the same glass still and used to make up the culture solution. These results indicate a slight accel-

eration of growth by dilute heavy water, but hardly beyond the limits of experimental error.

Chlamydomonas. The culture solution used contained KH_2PO_4, 0·7 gm.; $NaNO_3$, 0·7 gm.; $MgSO_4$, 0·2 gm.; NaCl, 0·07 gm.; $CaCl_2$, 0·07 gm.; and a trace of iron in 1,000 c.c. An old pure culture was well shaken, filtered through gauze, and the experiment and control inoculated with equal quantities of the organism. After four weeks exposure to diffuse light, the number of organisms was counted in a unit volume with the following results:

Experimental procedure.	No. of Organisms.
A. In original heavy water (1: 1,000)	7,300
In distilled water	10,900
B. In glass distilled heavy water (1: 1,000)	27,900
In glass distilled water	17,900

In this case using similarly distilled samples, heavy water appears to benefit growth appreciably.

Phormidium autumnale. For this blue-green alga the same culture solutions were used as in the preceding experiment, and heavy water and distilled water redistilled in the same glass still. The cultures were infected with equal quantities of pure cultures of *Phormodium*, and after four months the cultures were filtered, dried, and weighed. The heavy water cultures contained more organisms and were deeper in colour than the controls.

	1 per 1,000 heavy water.	Glass distilled water.
Average dry weight	0·04 gm.	0·012 gm.

NON-CHLOROPHYLLOUS PLANTS.

Fungi. Richards' culture solution was used containing 5 p.c. of cane sugar, and both waters were redistilled in the same glass still. The culture solutions, after sterilizing, were infected with equal quantities of spores, kept for 3 months at 25°C., then filtered, dried, and weighed.

Experimental procedure.	Average dry weight.
1. *Rhizopus nigricans.*	
A. In heavy water (1: 1,000)	0·055 gm.
In glass distilled water	0·1 „
2. *Penicillium citrinum.*	
B. Using spores. In heavy water (1: 1,000)	0·29 „
Using spores. In glass distilled water	0·29 „
C. Using mycelium. In heavy water (1: 1,000)	0·233 „
Using mycelium. In distilled water	0·288 „

In the experiment with *Penicillium citrinum* (B) although the dry weights were the same, spores developed earlier in the heavy water cultures. As a delay in the germination of the spores would affect the result, it was checked (C) by adding disks of mycelium 9 mm. diameter cut from plate cultures to fresh flask cultures. In both fungi a slight depressant influence on growth is shown.

Bacteria. Bacillus cloacae was taken as representative of the group of non-sporing bacilli, and of the sporers *Bacillus subtilis* was found a convenient form to use.

The method followed was to inoculate 10 c.c. of ordinary distilled water and 10 c.c. of heavy water, respectively, with a loopful (3 mm. × 1·5 mm.) of a 24 hour broth culture of the organism. Both samples of water were previously sterilized by autoclaving at 1½ atmospheres pressure for approximately 10 minutes; and after inoculation they were incubated at 30°C. for 24 hours. Each water culture was then plated out in 1 c.c. lots, and after pouring with ordinary nutrient agar these plates were incubated at 25°C. for 24 hours. The number of colonies which appeared was noted, and the results are given below:

Date.	Organism.	Type of Water.	Plate I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	Average Count.
April 19th	*Bacillus cloacae*	Heavy	1	2	1	0	0	5	2	7	—	2
		Ordinary	54	90	90	64	119	196	61	161	—	104
June 9th	*Bacillus cloacae*	Heavy	65	54	89	42	87	83	25	84	—	66
		Ordinary	160	115	72	94	122	90	192	189	—	129
June 21st	*Bacillus subtilis*	Heavy	0	3	0	1	0	0	0	0	0	—
		Ordinary	9	20	25	90	20	11	1	16	26	24
July 6th	*Sarcina lutea*	Heavy	0	178	83	59	122	156	84	217	269	129
		Ordinary	50	230	189	267	223	146	221	247	26	177

The concentration of heavy water used on April 19th was of 1 in 1,000 parts, also the heavy and ordinary water were not distilled under exactly similar conditions.

The water used in subsequent experiments was all distilled under identical conditions and the strength of heavy water was 1·3 parts per 1,000. Dilute heavy water strongly retards the growth of both sporing and non-sporing bacilli, but has less effect on *Sarcina lutea*.

Yeast. For this purpose a pure culture of a bottom yeast, *Saccharomyces cerevisiae*, was supplied by the Carlton and United Breweries, Ltd. The method was as for bacteria, but in order to meet the nutritive requirements of this fungal form, nitrogenous nutrient broth and agar were replaced by media rich in sugar. The inoculum was one loopful (approximately 1 mm. in diameter) of a 24 hour culture of the organism growing in a 1 p.c. saccharose solution. The plates were poured with 2 p.c. malt extract agar.

The yeast colonies were more tardy in appearing than was the case with the bacteria, and an incubation period of 72 hours at 25°C. was necessary before the following count could be made.

Date.	Organism.	Type of Water.	Plate I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	Average Count.
July 14th	*Saccharomyces cerevisiae*	Heavy	17	15	22	33	34	22	20	31	44	26
		Ordinary	20	18	17	28	16	24	25	22	26	21

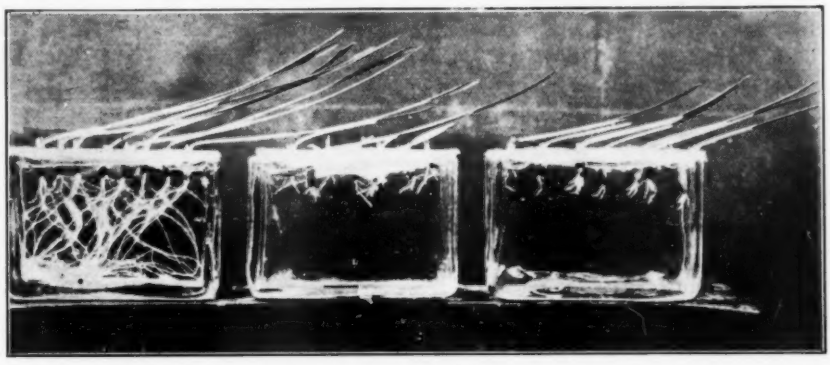
Apparently dilute heavy water distinctly retards the growth of the bacteria used but has little effect on that of yeast in the concentration used beyond a slight but fairly consistent acceleration.

THE EFFECT OF DILUTE HEAVY WATER ON THE GERMINATION OF OAT SEEDLINGS, *AVENA SATIVA*, ALGERIAN OATS, AND THE RESPONSE OF THE COLEOPTILES TO WHITE LIGHT.

Method. Three small straight-sided glass vessels were filled with (1) tap water; (2) heavy water, 1·3 per 1,000; (3) glass distilled water; and covered with cotton gauze dipped in paraffin

wax. Eight oat seedlings were inserted in each, and the vessels were placed side by side in a dark box, and subjected to unilateral white light from a 200 watt daylight globe. The whole apparatus was placed in a dark room in the centre of the building, so that the temperature remained practically constant. The insertion of a water filter at the aperture of the box removed heat-waves, and prevented the negative thermotropic reaction of oat coleoptiles. As the experiment continued, a certain amount of evaporation occurred, and the level was maintained by adding more water.

The experiment was commenced on April 20th; five days later the vessels were removed from the box to a dark room, and placed in series for photography. The vessels were replaced in parallel series in the dark box, and rephotographed on April 26th. A later photograph was taken on May 1st to show the development of the first leaves and the root systems. (Fig. 1.)



1. Tap water. 2. Heavy water. 3. Glass distilled water.

The table of germination is as follows, the temperature in the box remaining practically constant at 16°C.

NUMBER OF SEEDS GERMINATED.

	April 25th.	April 26th.	April 27th.	April 28th.	April 30th.	May 1st.	May 2nd.
Tap water	8	—	—	—	—	—	—
Heavy water	2	3	4	4	4	6	8
Glass distilled	3	5	8	—	—	—	—

The only effect noticeable here is that heavy water in a concentration of 1·3 per 1,000 delays the germination of oat seedlings, but once germinated their phototropic response does not appear to be affected. To test this more fully, Algerian oats were germinated in (a) heavy water, (b) glass distilled water in the dark room. Pairs of seedlings each of equal length—from 2-5 cm.—were chosen, one from glass-distilled water, and the other from heavy water, and were subjected to unilateral white light from a 200 watt lamp one metre away. As each pair of seedlings used for comparison was under the same conditions, little further control of the external conditions was necessary. All experiments were carried

out in a dark room, and the temperature throughout the series remained practically constant but was below the optimum (25°C.) for such experiments.

One series of experiments was carried out with the aid of a Klinostat. Four pairs of seedlings were successively fixed on to the Klinostat, stimulated with unilateral light for five minutes, and then rotated. In each case curvature was noticeable to the naked eye within an hour and was pronounced within six hours, but the seedlings from both the heavy water and the glass-distilled water responded equally. Further experiments were carried out without the Klinostat, and the tips of the seedlings were examined through a horizontal microscope in order to obtain the reaction time.

After unilateral illumination for five minutes the first visible curvature occurred in about 10 minutes, and here again no constant difference in the reaction times was obtained for each type of seedling. The examination of the seedlings was made in red light which had been proved to have no effect in promoting curvature in oat coleoptiles.

A further experiment was carried out subjecting pairs of seedlings to intermittent light in order that any differences would be exaggerated, but no noticeable difference in the time taken for each seedling to react was noticed. Hence the saturation of a seedling with water containing 1·3 per 1,000 of heavy water does not affect its heliotropic response.

Even dilute heavy water may, however, exercise a direct action on movement. The small water snail *Ancylus tasmanicus* has a limpet-like shell but it is flatter, ellipsoid, 2-3 mm. long and 1-2 mm. broad, cartilaginous and transparent. The snails feed on the minute algae on the glass walls of the tanks, and hence can be kept under close observation with the aid of a horizontal microscope. The water from some of the tanks was removed, rapidly evaporated down to a few drops, made up to the original volume with 1·3 per 1,000 heavy water and replaced in the same tanks. In 2-3 days the snails were distinctly more sluggish, and in three weeks movement had practically ceased. On refilling the tanks with tap-water, within three days the snails were crawling over the glass and feeding.

SUMMARY.

Heavy water even in low concentration (1 to 1·3 per 1,000) is capable of exercising a distinct physiological influence in experiments conducted under proper control. In non-chlorophyllous organisms a slight to distinct retarding action is, in general, exercised on movement and growth, the latter very markedly so in the case of bacteria, whereas spore production may be accelerated.

In the case of chlorophyllous plants, however, dilute solutions of heavy water appear to accelerate growth, and although the effect is slight in some cases, as in *Lemna*, stronger solutions might exercise a more pronounced action. If Washburn and Smith (1934) are correct in stating that growing willows exercise a

preferential selective absorption of heavy water, it is possible that heavy water may be preferentially used in the production of carbohydrates by photosynthesis, and that such carbohydrates may accelerate growth. If this were so, then the action of heavy water on green plants would be the resultant of two opposite actions: (1) the general depressant action as evidenced by non-chlorophyllous plants, and (2) the accelerating action due to heavy water favouring photosynthesis or producing more active or more plastic carbohydrates. The very slight accelerating action on the growth of yeast may be due to the fact that carbohydrate metabolism is the dominating factor in the life of this organism. Dilute heavy water retards the germination of oats, but at this stage the plant is non-chlorophyllous and is using previously stored food materials. Although the oat seedling was used as a non-chlorophyllous organism, dilute heavy water appeared to exercise a slight beneficial action upon its growth. This may be due to the fact that carbohydrate metabolism is a dominating factor in the nutrition of this plant. Heliotropic curvature is not affected by heavy water in a concentration of 1·3 per 1,000 but it is quite possible that stronger solutions might exercise a retarding influence.

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ANTIGENIC DIFFERENCES BETWEEN THE VENOMS OF THE TIGER SNAKE *NOTECHIS SCUTATIS* AND THE BLACK TIGER SNAKE *NOTECHIS SCUTATIS VAR. NIGER*

by

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In an earlier study (Kellaway and Thomson, 1932) it was shown that the melanotic tiger snake does not differ sufficiently in morphological characters from the typical banded form to warrant the erection of a new species, but comparison of the venoms revealed differences greater than any previously observed between individuals of any other species of Australian snake.

The venom of the melanotic form is less toxic than that of the banded tiger snake when injected subcutaneously, but has a more potent coagulant action both *in vitro* and when injected intravenously. The venoms are about equal in haemolytic power and in "curarizing" action, but in the neutralization of the venoms by monovalent antivenene, prepared against the venom of the typical banded form, striking differences are displayed.

Holden's (1933) success in preparing fractions of both these venoms almost free from thrombin has afforded opportunity for further study of the antigenic differences between these two venoms.

1. *Passive protection by univalent antivenene prepared by injection of the venom of the typical banded form.*

In Table I is set out a series of observations on the passive protection afforded by a dose of 0·5 c.c. of monovalent tiger snake antivenene prepared by Dr. F. G. Morgan at the Commonwealth Serum Laboratories. The antivenene was mixed with varying doses of the venoms and fractions, and the mixtures were allowed to stand for an hour at room temperature (19° C.) before injection into guinea pigs.

This monovalent antivenene in a dose of 1·0 c.c. neutralized approximately 0·4 mg. of tiger snake venom, 0·206 mg. of the corresponding thrombin free fraction, 0·296 mg. of black tiger snake venom, and 0·156 mg. of the corresponding thrombin free fraction.

TABLE 1.

Neutralization by Monovalent Antivenene of Tiger Snake and Black Tiger Snake Venoms and of Fractions Freed from Thrombin.

Weight of guinea pig in gm.	Dose of venom or fraction in mg.	Result.
Tiger venom.		
327	0·22	Died on the first day.
370	0·20	Survived without symptoms.
Tiger venom fraction.		
327	0·12	Died in less than 18 hours.
350	0·11	Died on the second day.
360	0·10	Survived without symptoms.
Black tiger venom.		
368	0·20	Died in 4 hours.
365	0·17	Died during the first day.
350	0·16	Survived without symptoms.
Black tiger venom fraction.		
393	0·11	Died in 6 hours.
395	0·1	Died in less than 18 hours.
364	0·1	Died in less than 18 hours.
385	0·09	Survived without symptoms.

For the guinea pig, the subcutaneous lethal doses of the two fractions are the same as those of the whole venoms—0·002 mg. for tiger snake, and 0·006 mg. for black tiger snake, per 100 gm. The more powerful coagulant activity of black tiger snake venom does not account for its less potent action when injected subcutaneously. Expressing the above results in terms of the lethal dosage per 100 gm. of guinea pig, 1·0 c.c. of the antivenene neutralizes about 200 doses of tiger snake venom, 103 of the thrombin free fraction of tiger snake venom, 49 of black tiger snake venom, and 26 of the thrombin free fraction of black tiger snake venom. Not enough of this sample of antivenene was available to ascertain its protective value against other venoms. It was found that 1·0 c.c. of another sample neutralized 0·327 mg. of tiger snake venom, 0·24 mg. of black tiger snake venom, 0·15 mg. of cobra venom, 0·10 mg. of copperhead venom, 0·032 mg. of brown snake venom, 0·025 mg. of death adder venom. This antivenene neutralized only an insignificant amount of black snake venom since 2·0 c.c. failed to protect guinea pigs of 300 gm. when mixed with a single lethal dose. Expressing these results in terms of lethal dosage per 100 gm. of guinea pig, this second sample of antivenene neutralizes 163 lethal doses of tiger snake venom, 40 of black tiger snake venom, 17 of copperhead venom, 13 of brown snake venom, 3 of cobra venom, and 1·6 of death adder venom.

2. *Active immunization with the thrombin free fractions of tiger snake and black tiger snake venoms.*

Two groups, each of two dozen rabbits, were immunized by the subcutaneous injection of the thrombin free fract' as of the venoms of the tiger snake and black snake prepared by Mr. H. F. Holden. Immunization was commenced on 28/2/33 with a dosage of 0·01 mg. in normal saline. Injections were given twice weekly until 11/12/33, by which time the dose had reached 0·55 mg. Injections were given thereafter weekly until 15/6/34 without much increase in dosage. After this latter date the dosage was increased, reaching 1·0 mg. on 20/7/34 for black tiger snake venom fraction, and 1·4 mg. on 10/8/34 for tiger snake venom fraction. The larger doses were given in sodium sulphate to exclude the toxic action of any traces of barium which might be present in the fractions.

In the group receiving tiger snake fraction there were five deaths in the course of immunization after doses of 0·28, 0·5, 0·56, 1·2, and 1·4 mg. In that receiving black tiger snake fraction there were ten deaths during immunization following doses of 0·038, 0·17, 0·5, 0·5, 0·55, 0·56, 0·575, 0·7, 0·8, and 0·8 mg.

Growth curves constructed from the average weights of the 20 rabbits immunized with tiger venom fraction and the 17 rabbits immunized with black tiger snake fraction, which survived till May, 1934, showed a steady increase in weight from 1·5 kg. in February, 1933, to 2·7 and 2·8 kg., respectively, in September, 1933. From this time till May, 1934, both curves were flat, that for the tiger snake fraction rabbits remaining at a slightly lower level.

Previous experience (Kellaway, 1930) rendered it unlikely that an investigation of the active immunity of these animals would be of much use in indicating antigenic differences between black tiger snake and tiger snake venoms, since striking degrees of cross protection had been observed between the venoms of the tiger snake and copperhead in guinea pigs immunized with these venoms; and guinea pigs actively immune to two lethal doses of death adder venom had been resistant to 8 and 10 lethal doses respectively of copperhead and tiger snake venoms. In any case the number of animals available at the end of immunization was wholly inadequate for estimation of the degree of active immunity present to the whole venoms and their fractions. A few observations were made which give some indication of the extent to which a fraction affords protection against the whole venoms.

Rabbits immunized by injection of tiger snake fraction survived without symptoms the subcutaneous injection of 6, 8, 14, 18, and 20 certainly lethal doses of tiger snake venom. A rabbit weighing 2·3 kg. which received 22 lethal doses (2·3 mg.), and another weighing 2·2 kg. which received 28 lethal doses (2·77 mg.), both died on the second day. Another rabbit weighing 2·9 kg. received 24 lethal doses (3·13 mg.), and died 12 minutes later with whipped out fibrin in the right heart.

Others of this group of immune rabbits were tested by subcutaneous injection of black tiger snake venom. Two survived without symptoms doses of 7 and 10 lethal doses, one weighing 2·87 kg. received 4·3 mg. (15 lethal doses), and died on the second day, but another weighing 3·05 kg. survived without symptoms a dose of 6·1 mg. (approximately 20 lethal doses).

Two rabbits withstood without symptoms the injection of 1 mg. per kg. of black tiger snake fraction after three had survived 0·5, 0·6, and 0·7 mg. per kg.

These rabbits which were immune to between 0·5 and 0·7 mg. per kg. of tiger snake fraction were immune also to more than 1·0 mg. per kg. of black

tiger snake fraction, to 0·9 mg. per kg. of tiger snake venom and to between 1·0 and 2·0 mg. of black tiger snake venom.

Similar experiments with rabbits immune to black tiger snake fraction showed that these were immune to at least 1·2 mg. per kg. of the corresponding whole venom.

These animals immunised with the fractions could be rendered immune to at least 28 lethal doses of either of the whole venoms within a few weeks.

The protocol of Rabbit 17 is typical.

Rabbit No. 17, which had been immunized with black tiger snake fraction (highest dose 0·575 mg.) and which weighed at this time 2·7 kg., received subcutaneously on 20/3/34 0·85 mg. of tiger snake venom (7 lethal doses), on 27/4/34 1.20 mg. (10 lethal doses), on 4/5/34 1·44 mg. (12 lethal doses), on 18/5/34 2.23 mg. (18 lethal doses), on 25/5/34 2·77 mg. (22 lethal doses), and on 1/6/34 3·53 mg. (28 lethal doses) of tiger snake venom. During this course of injections no symptoms of envenomation were observed, and the animal's weight increased to 2·8 kg.

3. *Titration of the Neutralizing Power of the Sera of Rabbits Immunized with Thrombin Free Fractions of the Venoms.*

Three separate sets of experiments were made. In the first we used pooled sera from all the rabbits of each of the two groups bled on 15/3/34, six days after the injection of a dose of 0·5 mg. In two further sets of experiments we pooled the sera of four rabbits of each group which were bled on 6/6/34 and 31/7/34, four and five days respectively after the injection of doses of 0·5 mg. of the fractions.

The amounts of the venoms and of the corresponding thrombin free fractions neutralized by 1·0 c.c. of the pooled sera used in each experiment, together with their equivalents in certainly lethal doses per 100 gm. of guinea pig are set out in Table 2.

TABLE 2.

Antibody Titre of the Sera of Rabbits Immunized with Thrombin Free Fractions of the Venoms of the Tiger Snake and Black Tiger Snake.

	1st Series.		*2nd Series.*		*3rd Series.*	
	Mg. of venom or fraction.	Number of C.L.Ds. per 100 gm. guinea pig.	Mg. of venom or fraction.	Number of C.L.Ds. per 100 gm. guinea pig.	Mg. of venom or fraction.	Number of C.L.Ds. per 100 gm. guinea pig.
Rabbits immunized with thrombin free fraction of tiger snake venom.						
Tiger snake venom	0·095	47·5	0·093	46·5	0·093	46·5
Thrombin free fraction of tiger snake venom.	0·084	42	0·094	47	0·122	61
Black tiger snake venom	0·155	26	—	—	—	—
Thrombin free fraction of black tiger snake venom.	0·046	7·6	0·038	6	0·058	9
Rabbits immunized with thrombin free fraction of black tiger snake venom.						
Tiger snake venom	0·075	37·5	—	—	—	—
Thrombin free fraction of tiger snake venom.	0·044	22	0·054	27	0·043	21
Black tiger snake venom	0·185	31	0·152	25	0·115	19
Thrombin free fraction of black tiger snake venom.	0·054	9	0·070	12	0·057	9

The figures obtained in these three series show fair correspondence though they are not strictly comparable. In the last two series the rabbits used were not all the same. In comparison with their protective power against whole tiger snake venom the sera of rabbits immunized with the thrombin free fraction of this venom give a higher proportional protection against this fraction and against black tiger snake venom than does Dr. Morgan's antivenene prepared against the whole venom. The sera of rabbits immunized with black tiger snake venom fraction give a relatively high degree of protcetion against whole tiger venom and its thrombin free fraction.

Apart from any antigenic differences due to the thrombins of the whole venoms, the thrombin free fractions are themselves antigenically different. The sera of rabbits immunized by injections of tiger snake venom fraction protect against about half as much of the fraction of black tiger snake venom as of that used for immunization. Conversely the sera of rabbits immunized by injections of black tiger snake fraction protect against about four-fifths as much of the fraction of tiger snake as that of black tiger snake venom. Comparison based upon the lethal dose per 100 gm. of guinea pig emphasises this antigenic difference still further.

The additional experiments reported here indicate that the antigenic relationship between these two venoms is closer than the relationship between the venom of the tiger snake and that of any other of the Australian *colubridae*. The less toxic nature of the venom of the melanotic form as judged by subcutaneous injection does not depend upon the greater potency of its thrombin. Such antigenic differences as exist between the two venoms are also independent of the thrombins, being displayed by the thrombin free fractions. The venoms are too nearly alike antigenically to support the erection of a separate species for the melanotic form.

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ON THE ACTION OF PICRIC ACID ON GLOBIN AND HAEMOGLOBIN

by

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Recently it has been shown by Ishiyama (1928) that acids of different strength (hydrochloric acid, phosphoric acid, eosin, and taurine) do not combine with globin in equivalent amounts. On the other hand, however, every individual acid tested was found to combine with globin in an approximately constant amount. In the present communication the proportion in which picric acid combines with globin and haemoglobin is discussed.

(a) *The Action of Picric Acid on Globin.*

Globin was prepared according to the method of A. Hamsík (1930). Fresh citrated human blood is centrifuged and the corpuscles washed four times with 2 p.c. sodium acetate solution. After haemolyzing the corpuscles with two volumes of water, undissolved particles are removed by centrifugation. The haemoglobin is then coagulated with 1½ volumes of acetone and, after filtration, the moist coagulum is ground up with a 3 p.c. solution of oxalic acid in acetone. After standing from 10–20 minutes the extraction fluid containing most of the haemin is removed by filtration. The crude globin is then treated once more with the oxalic acid solution, then washed with acetone and ether, and finally dried in vacuo.

In a mortar about 5 gm. of the prepared globin are ground up with 50 c.c. of a cold, saturated aqueous solution of picric acid. Then in small portions 200 c.c. of a cold saturated alcoholic solution of picric acid are added while stirring and grinding. This mixture is allowed to stand for about an hour, and then the globin picrate formed is separated from the fluid by suction. The precipitate is then washed with a small amount of absolute alcohol, and then with benzol and ether till the wash ether remains colourless. With larger amounts it is advisable to grind up the precipitate with ether, in order to be certain that all excess picric acid has been removed. The globin picrate is finally dried in vacuo at about 60°C.

The globin picrate is a yellowish amorphous powder which is almost insoluble in ether, chloroform, and benzol. It dissolves in alkali and is partly precipitated as globin picrate by acids. Water added to the globin picrate, as well as alcohol, turns yellow owing to the solution and hydrolysis which take place. Large amounts of hot water completely decompose it into globin and picric acid.

(b) *The Action of Picric Acid on Haemoglobin.*

Cells from citrated or oxalated human blood are washed three or four times with physiological saline solution or a 2 p.c. solution of sodium acetate. They are then haemolyzed with about two volumes of water and the remaining solids are removed by centrifugalization.

To the haemolyzed cells a cold saturated alcoholic solution of picric acid is added slowly and under constant stirring in order to avoid the formation of clumps. In all, about four volumes of alcoholic picric acid solution are added to one volume of haemolyzed cells. The precipitate formed under the conditions described consists of small, greenish-yellow amorphous aggregates of a slightly sandy consistency. This mixture is allowed to stand for about one hour, and then the precipitate is filtered off by suction. The colour of the filtrate is reddish-brown, and it contains the haemin component in solution. The precipitate is then washed with a small amount of alcohol, and finally with benzol and ether till the wash fluid remains colourless. It is then dried in vacuo. This product resembles globin picrate, as obtained from globin and picric acid, to a very large extent, and, as will be shown by analysis, it is actually identical with globin picrate.

The precipitation of the globin picrate is practically quantitative, and only small amounts of the yellow amorphous material are obtained by concentrating the filtrate. However, on slow evaporation of the mother liquor, large brownish crystals are obtained which appear to be some form of the haemochromogen.

The precipitate, when further extracted with ether in a Soxhlet apparatus for several hours, yields slightly more of a brownish ether-soluble substance which apparently is also derived from the haemochromogen.

A similar product is obtained if one slowly adds a haemoglobin solution to a boiling alcoholic picric acid solution. In the hot solution no precipitation takes place, and this mixture may be filtered. On cooling, however, the amorphous globin picrate appears almost quantitatively. The precipitation with aqueous picric acid is less complete and the product obtained is more brownish in colour.

(c) *Analysis of the Reaction Products from Globin and Haemoglobin for their Picric Acid Content.*

The principle of the analysis is as follows:

Picric acid forms with methylene blue a methylene blue picrate which is sparingly soluble in water and considerably more soluble in chloroform. This reaction has been found to be suitable for the analysis of picric acid in inorganic and organic picrates (Bolliger, 1933, 1934).

In the case of globin picrate no serious interference from the globin may be expected, since it has been shown that methylene blue base does not combine to an appreciable amount with proteins, particularly when these are present in acid or neutral solution (Rawlins and Schmidt, 1929). As observed by us, this statement also applies to globin. At the same time, an excess of methylene blue is best avoided in the analysis.

The actual procedure is as follows: The yellow reaction product is dried in

vacuo, and is weighed out into a volumetric flask. Then warm sodium hydroxide, in approximately 0·5 p.c. solution, is added until complete solution has been effected. After cooling, the solution is acidified with about 0·5 p.c. sulphuric acid until a permanent opalescence or a small amount of precipitate occurs. At this stage the solution is practically neutral. A measured amount of 0·01N methylene blue is then added with stirring. The methylene blue picrate formed, as well as the globin, is precipitated to a large extent and the solution takes on a greenish-yellow colour. Methylene blue is added until the supernatant fluid has a distinct green or greenish-blue colour, but as mentioned before, an excess of methylene blue is best avoided.

The whole mixture is then made up with water to a known volume and is mixed well. Some of the fluid is then freed from the precipitate by centrifugalization or sedimentation. An aliquot part of the clear fluid is transferred to a separating funnel containing chloroform. After shaking, the methylene blue picrate still in solution will be extracted by the chloroform and the aqueous solution will be of a yellow colour, provided that no excess of methylene blue has been added. To the yellow aqueous layer 0·001N methylene blue is added from a burette until the layer becomes bluish and remains so after further extractions with fresh chloroform. (Bolliger, 1933.)

Example. In a 250 c.c. volumetric flask, 0·4078 gm. of globin picrate prepared from globin was dissolved in 40 c.c. of 0·5 p.c. sodium hydroxide solution with warming. After cooling, 0·5 p.c. sulphuric acid was added until a permanent turbidity appeared. 50 c.c. of 0·01N methylene blue were added to the solution, which was then made up to volume. Some of the solution was centrifuged, and 25 c.c. of the clear, supernatant fluid was transferred to the separatory funnel and titrated with 0·001N methylene blue. This required another 2·2 c.c. of 0·001N methylene blue. Altogether, therefore, there were required 52·2 c.c. of 0·01N methylene blue, which in the present instance had to be corrected by a factor 0·88:

$$\text{Picric acid} = \frac{52{\cdot}2 \times 0{\cdot}88 \times 100 \times 229}{0{\cdot}4078 \times 1{,}000} = 25{\cdot}7 \text{ p.c.}$$

Six determinations on two different samples of globin picrate prepared from globin gave a picric acid content varying from 25·3 p.c. up to 25·9 p.c., with a mean value of 25·6 p.c.

Four different preparations obtained by the addition of cold alcoholic picric acid to a haemoglobin solution gave picric acid values ranging from 25·4 p.c. to 26·1 p.c., with a mean value of 25·7 p.c. Two preparations obtained by adding haemoglobin solution to a boiling alcoholic picric acid solution with subsequent filtration gave values of 25·4 p.c. and 25·8 p.c., respectively.

DISCUSSION.

By a simple process a well-defined picrate of globin can be prepared from globin, as well as from haemoglobin. When using haemoglobin solutions most of the haemin remains in solution while the globin is precipitated as globin picrate.

The picric acid content of globin picrate obtained from globin as well as haemoglobin, as found in numerous determinations, agrees closely with the figures found by Ishiyama (1928) for the phosphoric acid content of globin phosphate.

He found 11·45 to 12·07 p.c. phosphoric acid, which would correspond to a picric acid content of 26·7 p.c. to 28.1 p.c. This result seems to be a fairly logical one inasmuch as the acidities of picric acid and phosphoric acid are of about the same magnitude. By using a similar technique to that used for the picrate, dinitrophenol, which is a considerably weaker acid, was found to combine with globin to an extent of only about 8 p.c.

As far as we are aware, globin picrate has not been studied previously. This may be due to analytical difficulties. However, the determination of the picric acid content of the globin picrate by means of methylene blue is rapid and sufficiently accurate. The methylene blue, on account of its strong basic properties, combines completely with the picric acid, replacing the less basic globin.

SUMMARY.

Globin and haemoglobin form a globin picrate of constant composition.

The picric acid content as evaluated by the methylene blue method was found to be 25·7 p.c. $\pm$ 0·4 p.c.

ACKNOWLEDGMENT.

The authors wish to thank Miss Dorothy Dark for valuable technical assistance.

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ADRENAL CORTEX AND LUTEINISATION

by

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(Submitted for publication 27th September, 1934.)

Several attempts have been made to establish a functional connection between the cortex of the adrenal gland and the gonads. The clinical findings in interrenalism and tumours of the cortex itself provide sufficient evidence of some relationship, but the experimental evidence, as might be expected from the complexity of the subject, is incomplete.

Our interest has been for some time directed to the elucidation of the action of the prolans—the two factors obtained by the method of Zondek from the urine of pregnant females—in both human subjects and experimental animals, and the present paper reports the result of four separate series of experimental attempts to establish one of the alleged cortico-gonadal connections.

Corey and Britton (1931) showed that injections of adrenal cortical extract made by the method of Pfiffner (1931), into white Wistar rats, caused precocious maturity at the age of 28 days, more particularly in the females, the changes being less evident in the male. The pituitary glands of these animals were shown to be hypertrophied as early as 20 days under this treatment, and it was deduced that the effect on the gonads was brought about through the intervention of the hypophysis. Britton, Flippin, Silvette, and Kline (1931) showed incidentally that oral administration of Pfiffner's extract could control the effects of adrenalectomy in the rat.

On the other hand, Müller (1931) using a cortical extract, the method of preparation of which is somewhat obscure, found that in both normal and thymectomized male rats sexual development was hindered, whilst "over-development of ova" and failure of luteinization were noted.

Martin (1932) found that adrenalectomy of white rats either supresses or causes irregularity in the oestrous cycle in 87·6 p.c. of cases, whilst intraovarial transplants of cortex protect the animals against such changes. The oxyphil cells, particularly of the anterior pituitary, are influenced by adrenalectomy, and deficient hormone production was assumed both because of these changes and the fact that injections of prolan, and also theelin, restored the oestrous cycle. The

adrenal deficiency was, however, unaltered by the injections, and the ovarian effect was therefore believed to be through the medium of the pituitary.

Gaunt (1933) found that the life-span of adrenalectomized rats was 7 days in 90 p.c. of rats from 4 colonies, whilst the 5th colony showed a 50 p.c. mortality only, in 30 days. These differences between colonies explain contradictory results obtained in experiments where survival plays a decisive role. Adrenalectomized rats under two months of age survive 5·7 days irrespective of sex. He showed that after loss of the adrenals the females rarely lactated sufficiently to rear young unless gross adrenal rests were present. Cleghorn (1932) showed that although the weight of young female mice injected with cortical extract increased 9 p.c. in 4 weeks, the age of maturation was not affected. He found also that the oestrous cycle of the adult mouse was uninfluenced by such injection.

Gaunt and Parkins (1932) found that immature rats and chickens after daily administration of 5–20 dog units of cortin for long periods showed no gonadal change. Winter, Reiss, and Balint (1934) attempted to avoid the obvious difficulty of extra adrenal cortical tissue by the following method. Immature rats of 30-40 grams were adrenalectomized, and within 12 hours of operating were injected with 1-30 rat units daily of sexual ripening hormone from pregnant urine, using controls from the same litter. Controls showed the usual follicular luteinization, together with the development of the secretory phase in the uterus. The operated animals did not form corpora lutea and the uterus remained in the proliferation stage. They assume from this that the cortex is essential to the formation of corpora lutea. It is noteworthy that they report wide differences between litters of animals in respect of resistance to adrenalectomy, and ascribe this to either variations in amount of accessory cortical tissue, or hereditary variations in resistivity.

The animals used for the present work were hooded rats (Mus Norvegicus), the period of development of which had previously been carefully determined by Matters and McKay (1934). The weights of the experimental animals varied from 35 gm. to 45 gm., and their ages from 30 to 42 days. We used the same method as that of Winter and his colleagues, the only difference being that we set out to establish the significance of cortical extract itself in the development of the corpus luteum. Four separate series of animals were used, in each of which adrenalectomized animals were divided into the following groups of four—A. receiving cortin plus prolan (Bayer); B. receiving prolan alone; C. animals not adrenalectomized, receiving prolan alone; D. non-adrenalectomized controls; E. adrenalectomized controls receiving neither prolan nor cortin, making a total of 48 adrenalectomized animals. Adrenalectomy was performed with a cautery through one medial skin incision and two muscular incisions over the poles of the kidneys. The results of the operation were remarkably good for such small animals; the very few deaths which occurred resulted shortly after operation, and were due either to haemorrhage or anaesthesic; otherwise the animals which died before the end of the 4-day period did so from adrenal deficiency in the non-cortin groups.

Daily Dosage: Cortical extract, ·2 cc. equivalent to ·05 gram cortex; Prolan (Bayer), ·2 cc. equivalent to 10 rat units.

The cortical extract not only sufficed to prevent signs of adrenal deficiency, but has been successfully used during 12 months in treatment of the crises of Addison's disease in two human cases. It was prepared by the method of Grollman and Firor (1934).

Examination of the sections in all four series shows without exception that the changes illustrated in the photomicrographs are representative of the whole. In the last series, vaginal smears indicated ovarian activity within 24 hours of the first injections of prolan. Macroscopically, the ovaries of animals receiving prolan and cortin were more hyperaemic and blood points were more obvious.

SUMMARY.

Four separate series of immature rats weighing between 35 and 45 grams, have been adrenalectomized and treated with adrenal cortical extract and prolan (Bayer), either alone or together, within 12 hours of operation.

Within four days of the start of the experiment the animals were killed and found to show extensive corpus luteum formation where either prolan alone or prolan plus cortical extract was administered.

Luteinization did not appear to be accelerated by the administration of cortical extract.

Prolan did not appear to protect animals from the effects of adrenal deficiency.

The adrenal cortex does not appear to be essential for luteinization so far as these experiments are concerned.

ACKNOWLEDGMENT.

We are indebted to the Department of Biochemistry in this University for the cortical extract used in this work.

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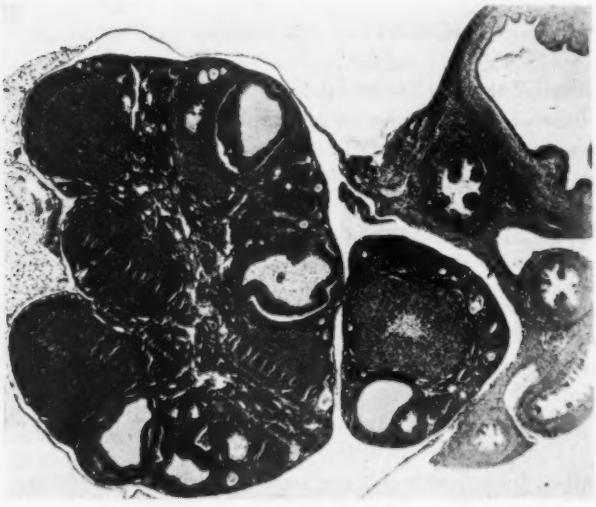
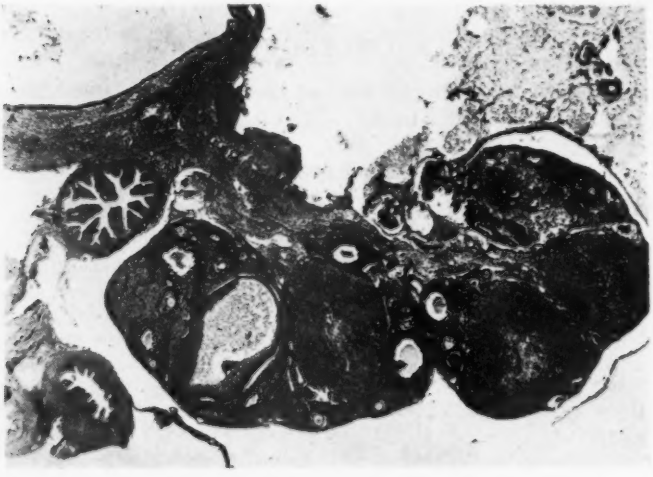


Group A. Ovary of adrenalectomized rat receiving prolan and cortin.



Group B. Ovary of adrenalectomized rat receiving prolan only.

Group C. Ovary of non-adrenalectomized rat receiving prolan.

Group D. Ovary of non-adrenalectomized control.

SOME OBSERVATIONS ON THE INORGANIC COMPOSITION OF WHEAT GROWN IN EASTERN AUSTRALIA

by

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(Submitted for publication 20th December, 1934.)

INTRODUCTION.

Very little information is available regarding the total ash content or the individual mineral constituents of wheat grown in Australia.

Kincaid (1911) investigated the phosphorus content of numerous plant materials including wheat. Her results indicate that certain plant materials grown in Australia are to some extent low in phosphorus in comparison with similar plant materials grown elsewhere.

Jewell (1932), (1934) has published figures on the total ash content of F.A.Q. (fair average quality) wheat grown in Victoria during the seasons 1926-27 to 1933-34 inclusive. His results, when calculated to a moisture free basis, showed that the average ash content of these particular samples was 1·56 p.c.

Coleman and his co-workers (1930) have published information regarding the milling and baking properties of wheat grown in various parts of the world, and have included in their results figures on the ash content of the wheat from every wheat-producing country. Their results for Australian wheat, obtained from 31 samples (collected from all states except Queensland), when calculated to the moisture-free basis, gave an average of 1·52 p.c. ash, which figure is similar to that found by Jewell for Victorian wheat. From Coleman's work it is possible to obtain a comparison of the average ash content of wheat derived from different countries. The following figures referring to wheat grown in 1926 and 1927 and calculated in all cases to the moisture-free basis were thus obtained:

Europe . . .	(142 samples)	1·92% ash
South America .	(11 „)	1·90% „
Africa . . .	(32 „)	1·86% „
Asia . . .	(50 „)	1·84% „
North America .	(73 „)	1·84% „
New Zealand .	(4 „)	1·80% „
U.S.S.R. . .	(40 „)	1·80% „
Australia . .	(31 „)	1·52% „

From these figures it is readily seen that the average ash content of Australian wheat is decidedly lower than that of wheat grown in other countries.

In order to obtain more information regarding the ash content of Australian wheats and to investigate the amounts of the various mineral constituents which they contain, a number of samples of wheat grown in Victoria and New South Wales have been collected and analysed.

METHODS OF ANALYSIS.

Each individual sample was cleaned, ground to pass a 30-mesh sieve, and analyzed in duplicate for the following:

Moisture: This was determined in a hot air oven kept at approximately 130°C. in accordance with the procedure adopted by the Association of Cereal Chemists (1928).

Ash: The sample treated with a glycerol-alcohol mixture was ashed in an electric furnace at low, red heat. Association of Cereal Chemists (1928).

Silica: This was determined in the usual way by dehydration of the hydrochloric acid solution of the ash. Association of Official Agricultural Chemists (1925).

Calcium and Magnesium: These were estimated in the silica free ash by the McCrudden (1909-10) method.

Phosphorus: A one-gram sample was ashed by the method of Howk and de Turk (1932), using calcium carbonate. Phosphorus was determined volumetrically in the resulting ash.

Potassium: The volumetric modification of the cobaltinitrite method was used. Milne (1929).

Manganese: This was estimated colorimetrically. Richards (1930).

Copper: This was determined colorimetrically by the Elvehjem and Lindow (1929) modification of the Biazzo method.

Iron: Stugart's (1931) method was used.

In addition the weight per 1,000 kernels was determined.

DISCUSSION OF RESULTS.

In Table 1, results of the above analyses are given for (i) 25 individual samples grown in different parts of Victoria, and (ii) 5 F.A.Q. samples from Victoria for the years 1924-25, 1925-26, 1930-31, 1931-32, and 1932-33. In Table 2 the ash content and the weight per 1,000 kernels (dry basis) are recorded for 22 individual samples from New South Wales. In each table the source and variety of the individual samples have been indicated.

Examination of Table 1 shows that there is a certain uniformity of ash content in the different wheats grown in the same district, but that there is some difference in ash contents of the wheats from the various districts (average figures for each district used in this comparison). For instance the ash content of the samples from the Central District is low in comparison with that obtained for the samples from the Wimmera District.

The average ash content of all the 25 individual Victorian samples is 1·38 p.c., which is lower than the average for the Victorian F.A.Q. samples for 1930-31 and 1931-32 (see Tables 1 and 3). This difference between the F.A.Q. sample and the individual samples grown during the same seasons is probably due to the

fact that some of these samples were not representative of Victorian wheat in general and were obtained from districts which are not large producers and therefore do not contribute greatly to the formation of the F.A.Q. samples.

TABLE 1.

The Inorganic Composition of Wheat Grown in Victoria.

District.	Year.	Variety.	Inorganic Composition of Wheat. In per cent. Ash.	SiO_2	Ca.	Mg.	K.	P.	In parts per million. Mn.	Cu.	Fe.	Weight kernels per 1,000
Central	1930	Free Gallipoli	1·22	0·016	0·019	0·115	0·317	0·249	48	7	62	41·4
"	1930	Free Gallipoli	1·19	0·015	0·030	0·117	0·315	0·248	57	8	37	37·7
"	1931	Free Gallipoli	1·39	0·029	0·031	0·136	0·452	0·242	67	5	59	34·3
"	1930	Nizam	1·12	0·015	0·031	0·108	0·340	0·185	40	6	46	40·0
"	1930	Nabawa	1·18	0·021	0·034	0·126	0·321	0·230	52	7	46	38·8
"	1930	Major	1·16	0·021	0·029	0·111	0·330	0·196	57	6	49	35·5
"		Average	1·21	0·019	0·029	0·119	0·346	0·225	53	6	49	37·9
Mallee	1931	Free Gallipoli	1·40	0·018	0·038	0·128	0·438	0·242	36	9	34	
"	1930	Ranee	1·29	0·017	0·050	0·134	0·269	0·249	41	6	58	40·7
"	1931	Ranee	1·26	0·019	0·043	0·118	0·366	0·237	50	5	32	
"	1931	Sepoy	1·42	0·025	0·035	0·130	0·350	0·257	35	5	41	35·7
"		Average	1·34	0·019	0·041	0·127	0·356	0·246	40	6	41	38·2
Goulburn Valley	1930	Free Gallipoli	1·20	0·015	0·038	0·107	0·366	0·262	39	10	41	46·2
" "	1930	Nizam	1·17	0·011	0·036	0·116	0·332	0·205	42	6	46	33·0
" "	1930	Nizam	1·49	0·022	0·037	0·131	0·412	0·310	53	6	44	38·3
" "	1930	Ranee	1·44	0·018	0·041	0·145	0·354	0·284	72	5	44	38·7
" "	1930	Comeback	1·32	0·016	0·031	0·135	0·322	0·283	82	8	62	31·9
" "	1930	Nabawa	1·53	0·021	0·038	0·133	0·410	0·303	66	7	58	39·1
" "	1930	Major	1·50	0·016	0·035	0·134	0·372	0·298	70	4	45	38·0
" "	1930	Minister	1·23	0·016	0·032	0·119	0·381	0·204	55	7	58	38·7
" "		Average	1·36	0·016	0·036	0·127	0·368	0·268	60	6	62	38·0
Wimmera	1930	Free Gallipoli	1·61	0·016	0·040	0·135	0·439	0·320	58	6	51	39·2
"	1930	Nizam	1·42	0·017	0·034	0·131	0·380	0·284	66	7	59	34·3
"	1930	Federation	1·47	0·021	0·036	0·141	0·433	0·269	41	7	49	35·2
"	1930	Comeback	1·67	0·018	0·036	0·158	0·351	0·362	49	4	51	31·4
"		Average	1·54	0·018	0·036	0·141	0·401	0·309	53	6	52	35·0
North Eastern	1931	Free Gallipoli	1·66	0·040	0·042	0·130	0·502	0·310	66	5	45	36·4
" "	1931	Federation	1·76	0·035	0·043	0·136	0·522	0·343	70	4	35	
" "		Average	1·71	0·038	0·043	0·133	0·512	0·327	68	5	40	
Western	1930	Free Gallipoli	1·41	0·012	0·021	0·120	0·390	0·288	67	6	44	36·8
		Average	1·38	0·019	0·035	0·128	0·378	0·268	55	6	47	37·2
Victoria	1924-25	F.A.Q.	1·62	0·019	0·039	0·145	0·432	0·304	47	8	62	34·8
"	1925-26	"	1·52	0·025	0·035	0·130	0·432	0·280	40	8	45	31·4
"	1930-31	"	1·62	0·028	0·044	0·150	0·428	0·326	46	8	57	34·6
"	1931-32	"	1·45	0·020	0·035	0·118	0·394	0·279	49	9	55	36·8
"	1932-33	"	1·58	0·029	0·037	0·141	0·497	0·262	58	4	43	

By the analysis of the F.A.Q. samples the average figures for Victorian wheats can be most easily obtained. The data available at present regarding the ash content of the F.A.Q. samples for the seasons 1924-25 to 1933-34 inclusive have been set out in Table 3. Some of these results were obtained in the present

investigation (see Table 1), and the remainder were derived from the published figures of Jewell which were recalculated to the same moisture-free basis. The average ash content of Victorian wheat for these ten seasons is 1·52 p.c., which value agrees very closely with that obtained by Coleman (1930) and his co-workers for Australian wheats for the season 1926-27. It should also be noted from Table 3 that there is some variation in the ash content of the F.A.Q. samples from year to year. The average ash content of the 22 samples of wheat grown in New South Wales was 1·47 p.c., a value not much below that obtained for the Victorian F.A.Q. samples.

TABLE 2.

The per cent. Ash and Weight per 1,000 Kernels of Wheat Grown in New South Wales, 1931.

Variety.	Source of Samples. Temora.		Wagga.		Trangie.		Cowra.		Condoblin.	
	% ash.	Weight 1,000 K.	% ash.	Weight 1,000 K.	% ash.	Weight 1 000 K.	% ash.	Weight 1,000 K.	% ash.	Weight 1,000 K.
Bobin	1·75	37·4	1·43	41·6	1·71	42·6	1·53	41·5	1·26	37·9
Waratah	1·55	37·9	1·41	39·6	1·52	34·5	1·48	44·0	1·31	35·2
Yandilla King	1·48	40·2	1·60	44·5	—	—	1·72	49·5	1·27	39·6
Nabawa	1·48	40·2	1·48	43·2	1·55	39·1	1·65	47·2	1·15	42·1
Canberra	—	—	1·29	38·8	1·65	35·2	1·47	39·4	1·16	36·8

TABLE 3.

Per cent. Ash in F.A.Q. Wheat Grown in Victoria.

Year.	Per cent. Ash.
1924-25	1·62†
1925-26	1·52†
1926-27	1·50*
1927-28	1·62*
1928-29	1·39*
1929-30	1·49*
1930-31	1·62†
	1·66*
1931-32	1·45†
	1·55*
1932-33	1·58†
	1·55*
1933-34	1·41†
	1·53*

* According to Jewell. † Determined by Author.

All these results substantiate the belief that the ash content of Australian wheat is lower than that of wheat grown in other countries. That this difference is a significant one can be readily seen on reference to Table 4, in which the frequency distribution of all results obtained for Australian wheats (results of the present investigation together with the results of Coleman and Jewell calculated to the same basis) have been recorded. For the sake of comparison Coleman's results for wheats grown in other parts of the world calculated to the same basis have been included in the same table.

TABLE 4.

Frequency Table.

p.c. ash (a).	Area considered. Australia (b).	North America (c).	Europe (c), including Russia.	Africa, Asia, South America, New Zealand, and Japan (c).
1·10–1·19	11	1	—	—
1·20–1·29	16	—	1	—
1·30–1·39	32	—	2	—
1·40–1·49	36	2	4	3
1·50–1·59	26	5	3	9
1·60–1·69	18	11	18	15
1·70–1·79	13	7	22	12
1·80–1·89	3	6	45	20
1·90–1·99	2	16	37	10
2·00–2·09	1	9	22	12
2·10–2·19	—	5	18	2
2·20–2·29	—	3	9	—
2·30–2·39	—	5	4	—
2·40–2·49	—	1	—	—
2·50–2·59	—	—	—	2
2·60–2·69	—	—	—	—
2·70–2·79	—	1	—	—
2·80–2·89	—	1	—	1
Total number samples	158	73	185	86

(a) Calculated where necessary to moisture-free basis.

(b) Figures include results given in Table 1 for Victorian wheat, in Table 2 for New South Wales wheat, by Coleman (1930) for Australian wheat, and certain unpublished figures on the ash content of wheat grown in Victoria in 1933.

(c) Figures obtained from the work of Coleman and co-workers.

There must be, therefore, some fundamental cause to account for the low ash content of Australian-grown wheat. This may be due to soil and climatic differences or to the fact that, in Australia, white wheats are those predominantly grown. Certain indications which bear on this point have been obtained, and they will be reported in another paper.

In this comparison with wheats from other countries, the percentages of the various mineral constituents have also been considered. If these mineral constituents are taken into account in the consideration of total ash, then the question arises as to their part in the general low ash content of Australian wheat. Is there a deficiency in one or more of these mineral constituents, which would help to account for the low total ash, or is each of the mineral constituents lower in amount in comparison with similar constituents of the wheat grown elsewhere? In order to elucidate this point and to investigate the relationship of each of the major mineral constituents, namely, phosphorus, calcium, potassium, and magnesium, to the total ash, the amount of each of these constituents of the wheat has been plotted against the total ash content of the same wheat. In the plotting of these values the logarithm of the constituent has

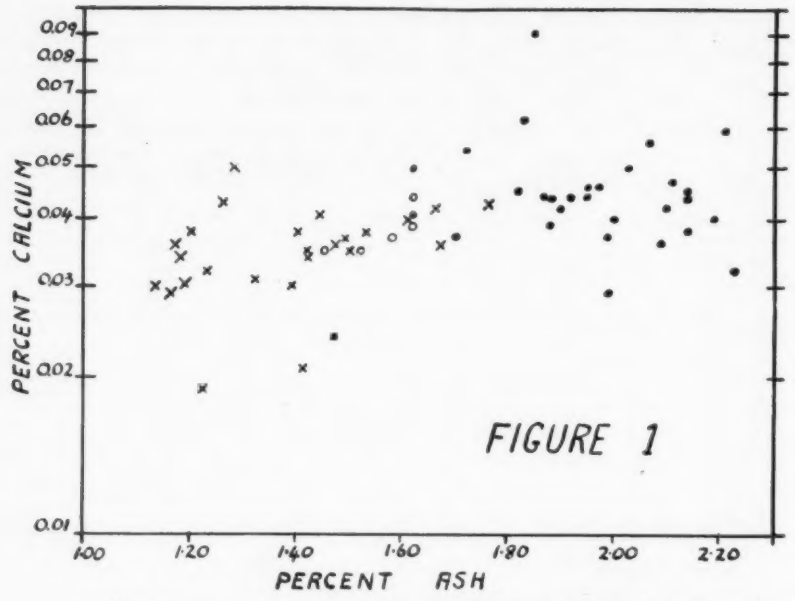


FIGURE 1

X-Victorian wheats : O-Victorian F.A.Q. wheat : ●-Overseas wheats

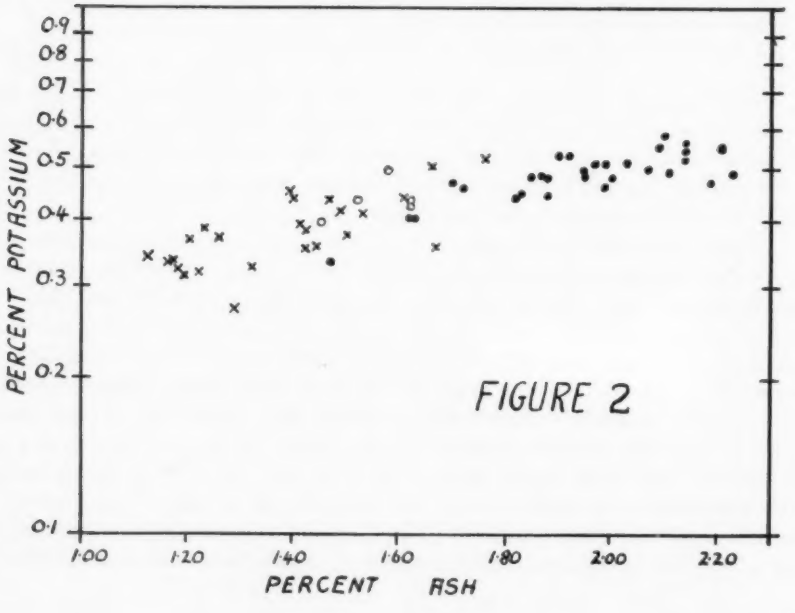
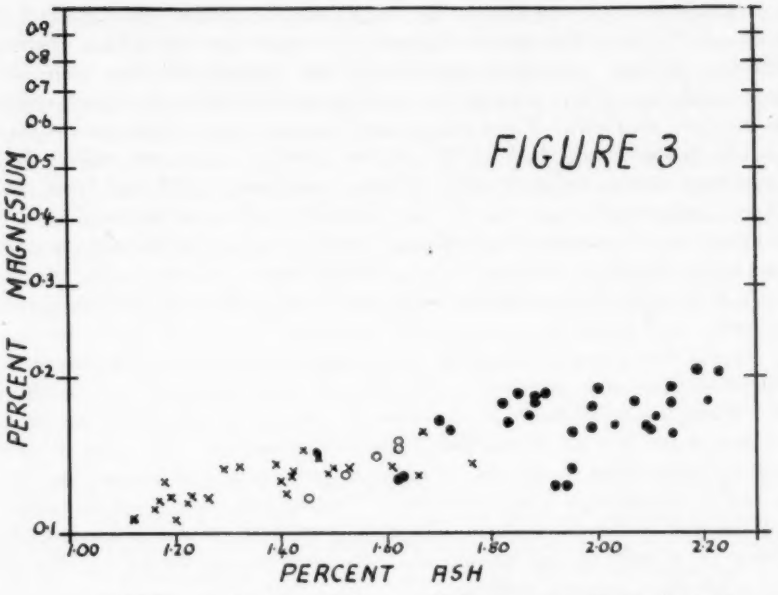
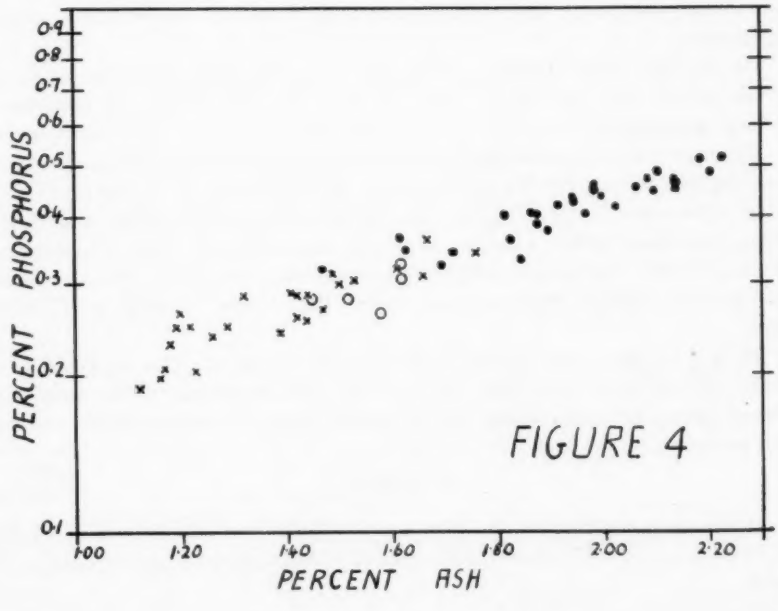


FIGURE 2



X- Victorian wheats : O-Victorian F.A.Q. wheat : ●- Overseas wheats



in each case been plotted against the ash on semi-log paper—see figures 1, 2, 3, and 4. At the same time and in the same figures the amount of each of the constituents, calcium, potassium, magnesium, and phosphorus, has been plotted against ash for various samples of wheat grown elsewhere for which complete analyses are available. These values have been obtained from various sources (namely, Greaves and Hirst (1929), König (1904), Lawes and Gilbert (1884), Richardson (1884), Snyder (1893), Sullivan and Near (1927), and Wolf (1871). Where necessary, the ash contents as recorded in these publications have been converted to the moisture-free basis, and the percentage of the various mineral constituents has been reported as the element instead of the oxide. From figures 1, 2, 3, and 4, it is possible to make direct comparisons and several interesting points may be noted.

In the first place considering both overseas and Australian wheats, it is found that there is an increase in each mineral constituent with increase in total ash. While this relationship is very close in the case of phosphorus, magnesium, and potassium, it is not so marked in the case of calcium. The rate of increase is of the same order in the case of calcium, potassium, and magnesium, but in the case of phosphorus it is more rapid. Thus an ash from wheat having a low total ash will contain a relatively lower proportion of phosphorus and a higher proportion of calcium, magnesium, and potassium than it would if it originated from a wheat of high ash content.

Since the wheats grown in Eastern Australia contain less total ash than those grown elsewhere, they will contain less of each of the principal mineral constituents.

On the basis of the figures 1, 2, 3, and 4, it is possible to predict in general the amount of each constituent, with the exception of calcium, on the basis of the total ash content.

The minor mineral constituents, namely, copper, iron, and manganese, have also been determined for Victorian-grown wheats (Table 1). There are no essential differences in these values from those reported by Elvehjem and Hart (1929), Headden (1915), Lindow, Elvehjem and Peterson (1929), Lindow and Peterson (1927), McHargue (1923), Peterson and Elvehjem (1928), Skinner and Peterson (1928), Sullivan and Near (1927), and Webster and Jansma (1929).

As will be seen from Tables 1 and 2 many of the samples analysed in the present investigation were also used for the determination of the weight per 1,000 kernels. No relationship was found between ash content and weight per 1,000 kernels.

SUMMARY.

It has been shown that the total ash content of wheats grown in Victoria and New South Wales is lower than that of wheats grown in other parts of the world.

Wheats from various districts have been analysed, and it has been found that there is a certain uniformity of ash content within a district. However, the ash content varies for wheats grown in different districts. It was also found that there is a variation in the ash content of Victorian F.A.Q. wheats for different seasons (samples taken over a 10-year period).

The Victorian wheats analysed contain, in general, smaller amounts of each of the major mineral constituents, calcium, potassium, phosphorus, and magnesium, than do wheats grown in other parts of the world. The composition of the ash from wheats having a low total ash varies slightly from that of wheats having a high total ash. This relationship has been shown diagrammatically.

Copper, iron, and manganese are present in Victorian wheats in approximately the same degree as in wheats grown elsewhere.

No relation was found between total ash content and the weight per 1,000 kernels.

ACKNOWLEDGMENTS.

The author is indebted to the University of Melbourne for a Research Grant; to Associate Professor W. J. Young for his interest and for making laboratory facilities available; to Professor S. M. Wadham for valuable suggestions and for assistance in the collection of samples; and to the Departments of Agriculture of Victoria and New South Wales for arranging for the collection of the various samples.

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NOTES ON SOME EXPERIMENTS WITH SNAIL TISSUE IN CULTURE

by

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(Submitted for publication 14th November, 1934.)

A number of experiments have been carried out with snail *(Helix)* tissue as described by Gatenby (1932).

Modifications of Gatenby's technique were used, and cultivation of the tissue, as far as possible, was carried out under aseptic conditions and with different types of culture media. In addition, attempts were made to demonstrate oxidases in the various cells by means of benzidine. Not only were pieces of the pulmonary cavity wall examined, but it was found that pieces of the snail's foot also responded very well to the "*in vitro*" technique, and the major portion of this work was carried out upon these preparations.

Generally speaking, the former work has been confirmed, but a number of interesting points have been observed which were not reported by Gatenby.

As with Gatenby's work no mitoses were seen, either in aseptic cultures or in cultures which were allowed to become badly infected; and several stages reminiscent of amitosis were observed.

A peculiar method of division which may be described as cytoplasmic fragmentation was observed in some of the cells, which may have been due to the formation of unusual pseudopodia.

The type of pseudopodial elongation observed by Gatenby was also characteristic of some of the cells in the cultures attempted by the present author, but a large number of cells did not show this tendency, and in addition it was frequently observed that epithelial cells of the foot tended to migrate *en masse*. The amoebocytes, which were in all cases the first cells to migrate, did possess very elongated filopodia.

Bulbous outgrowths, similar to those described by Gatenby, were found; but those in the pieces of cultivated foot were not so large as those which occurred in the pieces of explanted pulmonary wall. They appeared to be formed as a result of the stretching and disengaging of the cells in their endeavour to migrate, rather than to any actual cell division.

METHODS AND TECHNIQUE.

Following the removal of the shell from the snail, pieces of pulmonary wall were removed and placed into three types of culture medium, a drop of snail's blood, a saline extract of minced snail tissue, and sterile saline. In the first two preparations, migration proceeded slowly for a fortnight, when intensity of bacterial infection prevented further changes; this agrees with Gatenby's work. In the third preparation, migration continued slowly for a month without any extensive infection taking place. It was found that pieces of snail's foot treated in the same way gave a much better response, a far greater number of cells migrating within a comparable time.

The foot explant retained its power of movement for some days. During the first twenty-four hours it spasmodically curled up and returned to its normal shape at intervals of two or three minutes. In succeeding days these spasmodic movements became more infrequent, until after four days no further movement was observed. At this stage fairly extensive migration had taken place, and this gradual cessation of combined movement on the part of the cells was presumably due to the breaking up of the intercellular organization of the explant. The gradual breakdown of severed nervous tissue would be another factor associated with failure of combined movement after a lapse of time.

All the preparations were made using the standard hanging drop technique.

The foot explants, which were originally made (with aseptic precautions) into sterile saline (0·65 p.c.), were changed after a fortnight into a sterile mixture of commercial meat extract and 0·65 p.c. saline. In this medium the tissue remained for two months without any signs of degenerative changes in the cells, such as accumulation of fat, etc.

A further change of medium was made using the same mixture; and the transplant into this second supply of medium also showed no degenerative changes at the end of a further two months.

OBSERVATIONS ON THE CULTURES.

In view of the fact that Gatenby has dealt very fully with the culture of the pulmonary wall and that the present author's results confirm his observations, it is not proposed to give any detailed account of these cultures.

It was thought that the infected condition of some of Gatenby's original cultures may have been associated with the amitotic divisions which he found in numerous cells in his cultures. No mitoses were observed in the present author's cultures, whether they were aseptic or infected.

In the cultures of the pulmonary wall, a number of bulbous outgrowths were observed, and also small, round, pigment cells with a small sphere of pigment in the centre of the cell. In other cells the pigment took the form of a large sphere,

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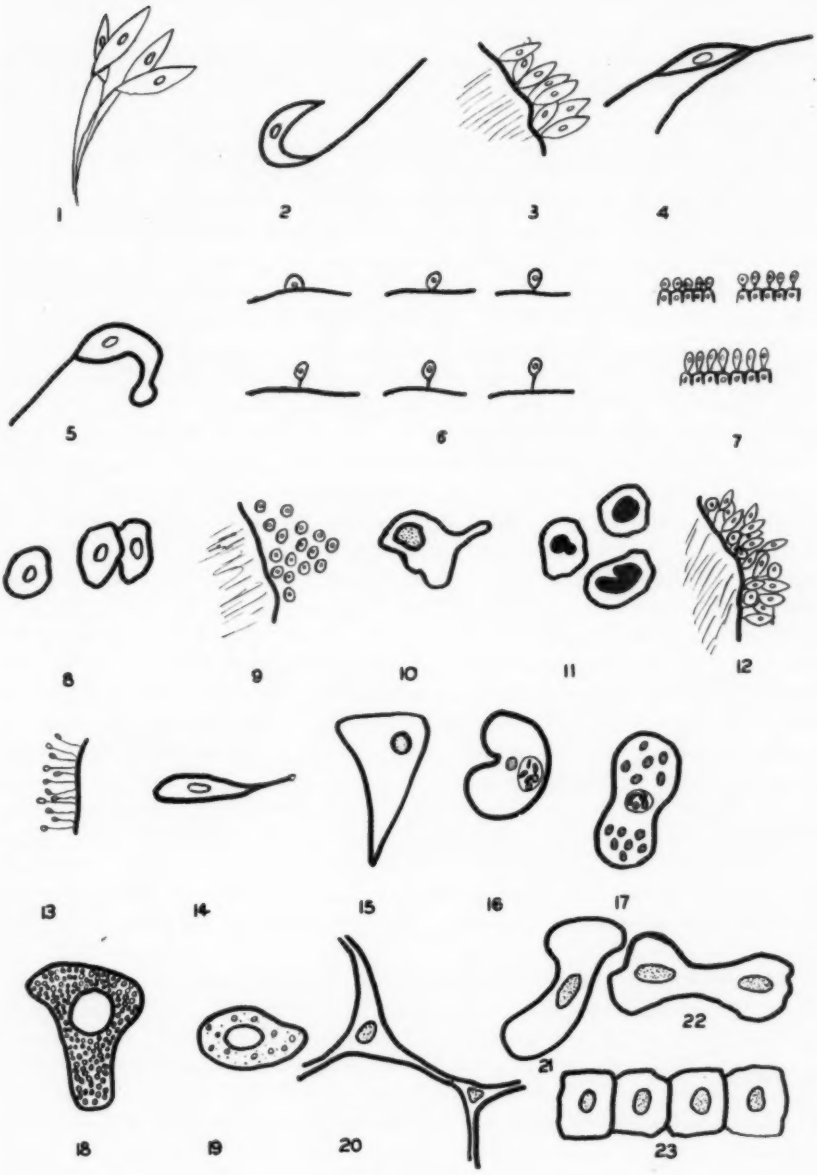
Figures 1, 2, 3, 4, and **5.** Migrating amoeboeytes. **6.** Method of migration of amoeboeytes. **7.** Migrating epithelial cells. **8.** Type of cells migrating from pulmonary wall. **9.** Unclassified cells from pulmonary wall. **10.** Types of pigment cell from pulmonary wall. **11.** Edge of explant showing migrating mass of cells. **13.** Cytoplasmic buds extending out from explant. **14.** A single cell showing a cytoplasmic bud. **15.** Form maintained by epithelial cells in culture. **16** and **17.** Oxidase in cells from pulmonary wall of snail. **18.** Migrated mucous gland cell. **19.** Cell containing a few mucous droplets. **20.** Cells from foot, showing the formation of filopodia. **21.** Another form taken up by epithelial cells. **22.** Apparent stage in cell division. **23.** Epithelial cells tended to flatten on the cover glass in this form.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or reniform mass, occupying the major portion of the cell. None of these cells were observed to put forth pseudopodia.

In all the cultures the first cells to migrate appeared to be amoebocytes. These were rather fusiform cells with a tendency towards attenuation at one or both ends.

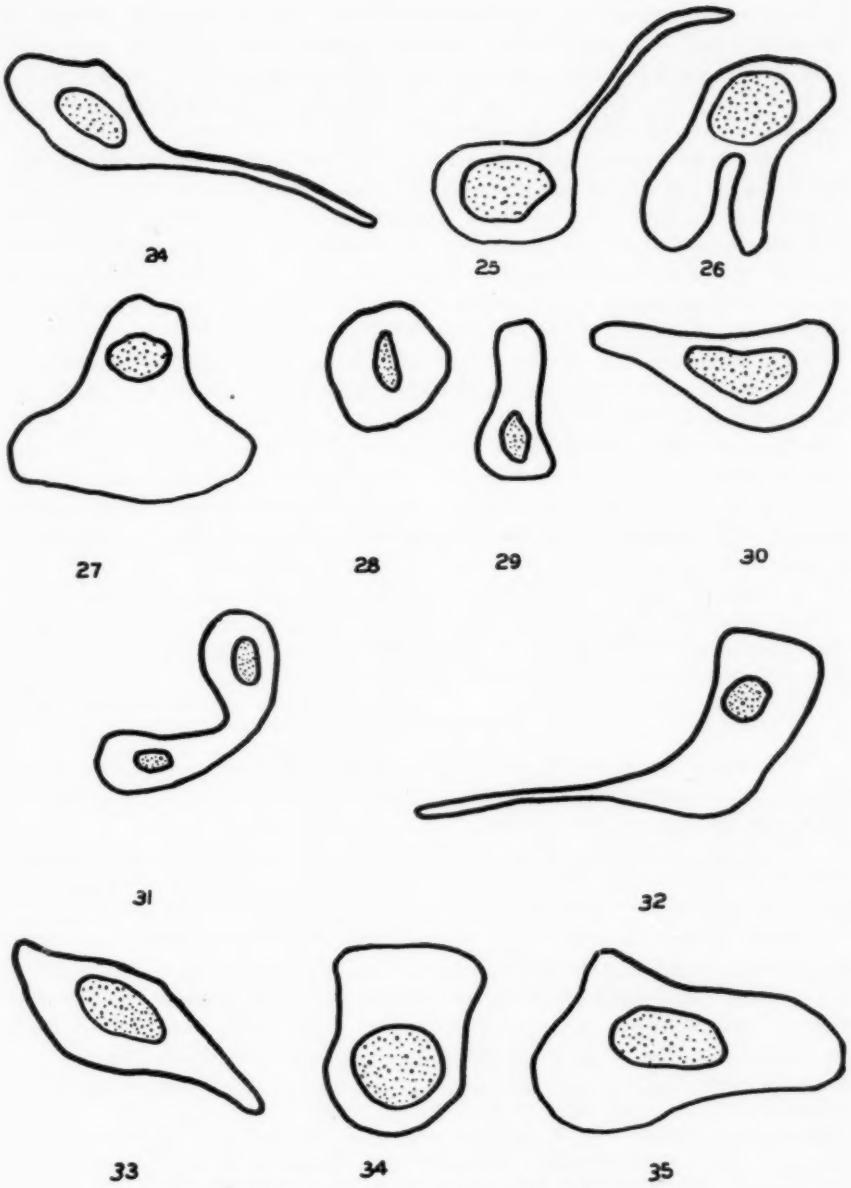
During migration a blunt end usually appeared, forcing its way out from the main mass of cells. This was followed by the cell body, and the cell finally emerged completely from the explant but remained attached by means of a long filopodium. After a few days (in the case of the foot explant) groups of these amoebocytes hung in clusters. In some amoebocytes a club-shaped protuberance was formed at the distal end, while in others it became filopodial; but in the great majority it remained blunt. At times the proximal connection became extremely long, and the cell appeared to be attached by a very thin thread.

Following the preliminary amoebocyte migration it was observed that masses of epithelial cells tended to separate away in a body. Some of the masses did not appear to have developed any pseudopodia, but others remained attached to the explant by means of long filiform extensions of the cytoplasm. The migration of these epithelial cells is illustrated in the figures. Extremely long filopodia were formed in certain of the cells, as if budding had occurred from portions of the cytoplasm. A cell exhibiting this form is shown in Figure 14. Before the migrated cells showing this form were observed, a bunch of these buds was discovered protruding from the explant (see Figure 13); and the author was at a loss to explain them, until the migrated cells exhibited this formation and it became apparent that they were a group of cytoplasmic buds. Horning (1932) has found that cells in culture undergo an unequal division of the cytoplasm following general hypertrophy of cytoplasmic inclusions. Horning assumes that this may indicate an attempt on the part of a degenerate cell to restore the nucleocytoplasmic ratio.

Horning's observations, however, apply to cells which were degenerating, whereas the cells described by the present author possessed no signs of degeneration; and in any case these buds were found quite early in the period of culture. Also it was not found to be more common as the cultures became older. The formation of these cytoplasmic buds must therefore remain at the moment inexplicable.

There did not appear to be any very great tendency for de-differentiation of cells to take place, as described by Horning and Richardson (1929) and others. After the cultures had been proceeding for some weeks, distortion of a few cells was apparent, and a number of cells was present which did not appear to maintain any special form.

The epithelial cells migrated with a flattened distal margin and a gradual attenuation of the proximal portion to a mere thread. This form was maintained right through the culture.



Figures 24-35. Varieties of cell shapes found in culture.

Some cells, apparently of a glandular nature, were observed in cultures of the snail's foot. They resembled migrated epithelial cells and were completely packed with small refractive globules, the nucleus being the only portion of the cell not occupied by them. Only a few of these cells were found, and they were regarded as having migrated from a mucous gland in the foot.

A few later stages of amitotic cell division were observed, but no recognizable early stages were seen in any of the preparations. In the cells which were just commencing to undergo cytoplasmic division the nuclei appeared well-formed, and did not appear to be reforming from chromosomes, which would have been the case if the nuclear division had been by mitosis. As no cells containing a spindle or chromosomes were observed it is assumed that the stages were those of amitosis.

Some of the unusual cell forms seen in culture are illustrated in figures 24-35.

In certain of the preparations of explanted foot attempts were made to demonstrate oxidase within the cell by means of benzidine. A number of bright blue particles was observed in the cytoplasm, and portions of the nucleus also gave the blue reaction. It thus appears that the nucleus contains oxidase, and this supports other work (unpublished) by the author on the presence of oxidase in the nuclei of certain pollen mother cells and in the germ cells of a number of nymphal Orthopterans.

A few cells were observed in which the only cytoplasmic region to give a definite blue colour was a rather large granule near the nucleus. This may be the idiozomic region of the cell, as Bles (1929) has found that centrosomes stain with oxidase reagents, as well as areas in the nucleus.

SUMMARY.

The author has been able to support the previous work on this subject by Gatenby and Duthie (1932), and has adduced further information using similar techniques on pieces of snail's foot.

The behaviour of the snail cells in culture appears to differ considerably from that of vertebrate cells under similar conditions, there being apparently very little tendency to exhibit the usual criteria of cellular degeneration.

A few of the cells assumed unusual shapes but the majority did not show any signs of de-differentiation.

Oxidase tests confirmed the present author's previous observations that the nucleus contains oxidase.

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AN ANALYSIS OF THE EFFECT OF PHOSPHORUS SUPPLY ON TRANSPIRATION RATIO IN PLANTS

by

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(Submitted for publication 13th February, 1935.)

INTRODUCTION

It has long been known that the transpiration ratio of plants is generally reduced by increase in nutrient supply. This effect was first analysed in physiological terms by Ballard (1933) for the case of nitrogen. He found that the reduction in transpiration ratio was due not to an effect on the transpiration rate per unit weight of leaf, but to an effect on growth.

The present paper has been devoted to an examination of the effect in the case of phosphorus. Richardson (1923) and Richardson and Trumble (1928) have shown for cereals and pasture plants that an increased supply of superphosphate reduces the transpiration ratio. No analysis of the effect in physiological terms, however, has yet been attempted.

THEORETICAL CONSIDERATIONS.

The transpiration ratio is the ratio of the amount of water transpired to the amount of dry matter formed during the whole or any part of the life-cycle. Ballard (1933) has derived the following equation for transpiration ratio:

$$\frac{y_T}{W_T} = \int_0^T \frac{W}{W_T} r dt, \qquad (1)$$

where y is the total amount of water lost between zero time and any other time t, W is the dry weight at time t, T is the value of t at harvest, and r is the rate of transpiration per unit dry weight of plant and is a function of t.

A more general expression applicable to data which do not commence from zero time may be obtained as follows:

$$\text{transpiration ratio} = \frac{y_{t_2} - y_{t_1}}{W_{t_2} - W_{t_1}},$$

and

$$dy/dt = Wr,$$

where the subscripts t_1 and t_2 indicate initial and final values of y and W for the interval over which the ratio is required, and the remaining symbols have the same significance as in equation (1).

Therefore

$$\frac{y_{t_2} - y_{t_1}}{W_{t_2} - W_{t_1}} = \int_{t_1}^{t_2} \frac{W}{W_{t_2} - W_{t_1}} r dt. \qquad (2)$$

In the experiments which are to be described accurate measurements of transpiration were not obtained during the 30 days following the planting of the seeds. For this reason equation (2) was used in the graphical analysis of the results.

It will at once be seen that, if W_{t_1} is small in comparison with W_{t_2}, the factor $W/(W_{t_2} - W_{t_1})$ becomes approximately equal to the growth factor, W/W_T, in Ballard's equation (1). Ballard neglected as inappreciable the water transpired during the first 23 days of his experiment.

A further important difference between the transpiration ratios of this paper and those of Ballard and many other workers is that the dry weight values include the roots as well as the shoots. When determined in this way the ratios clearly have a greater physiological significance.

MATERIALS AND METHODS.

On 7th June, 1933, seeds of a pure line of Algerian oats (*Avena sativa* Linn.) were planted in earthenware pots which had been filled with 14 kg. of water-washed sand.

The pots were divided into three treatment groups, and sodium dihydrogen phosphate was added in solution so that

Group	I received	0·046 gm.	NaH_2PO_4 per pot
Group	II "	0·603 gm.	NaH_2PO_4 "
Group	III "	2·418 gm.	NaH_2PO_4 "

In addition, all pots received the following nutrients in solution:

$Ca(NO_3)_2,4H_2O$	0·579 gm.	per pot
NH_4NO_3	0·754 "	" "
KCl	1·155 "	" "
$MgSO_4,7H_2O$	0·403 "	" "
$FeCl_3$	0·0302 "	" "
$MnSO_4,4H_2O$	0·0013 "	" "

The phosphate and the first three of the above nutrients were added on 17th June, and the remaining three on the 19th and 20th. Distilled water was then added so as to raise the water content of the sand to 2·015 l. which represented 55 per cent. of the maximum water-holding capacity of the sand. Weekly transpiration losses were recorded by weighing the pots and bringing them back to their previous weights by further additions of distilled water, and in order to minimize the loss by evaporation 1·5 kg. of fine gravel were spread on the surface of each pot. Six pots otherwise treated similarly were kept without plants so that allowance could be made for this loss by evaporation.

Harvests were taken on the 16th, 21st, and 26th June, 3rd and 17th July, 11th August, 7th September, 5th October, and 7th and 20th November. Only the last five harvests are of primary importance for the present paper, though dry weight values from the 4th and 5th harvests were

used in the determination of W_{t_1}. Harvests 5–10 were made by removing the plants from six pots of each treatment, each pot providing a replicate of three plants. At each of these harvests dry weight values were obtained for leaves, roots, and the whole plant. Prior to harvest 5 it was impracticable to obtain root weight values. Flowering occurred between harvests 8 and 9, and at harvest 10 the leaves of the plants of Treatments II and III were dead, whereas many of those of Treatment I were still green.

RESULTS.

Mean values of dry weight for the whole plant are presented in Table 1, and graphically in fig. 1. For harvests 6-10 these values constitute W_{t_2} in equation (2). Transpiration data commence at day 30 but, as no harvest was taken on that day, values for W_{t_1} were calculated from interpolations on the growth curves for the sub-aerial portions of the plant alone, on the assumption that the root : shoot ratios were the same as at harvest 5 (day 40).

The growth rate of plants of Treatment I was considerably less than that of Treatment II during the early part of the life-cycle, and, although subsequently it became greater, the final dry weight attained was little more than half that of Treatment II. There was also an initial depression of the growth rate of the plants of Treatment III; later, however, there were no significant differences in the results for Treatments II and III, and there is evidence, which need not be brought forward here, that nitrogen supply was a limiting factor in both cases. A detailed analysis of the effect of phosphorus supply on growth will be attempted on another occasion.

The transpiration ratios presented in Table 2 are means of six individual pot values. It has not been considered necessary to present the water loss data except graphically as mean cumulative water loss (fig. 7). The ninety individual values for the ratio were subjected to an analysis of variance, from which it was found that the effects of treatment as a whole (phosphorus and time interval) and of phosphorus alone are significant at the 1 per cent. point, and that the interaction of phosphorus treatment and time interval is significant at the 5 per cent. point. The standard deviation of the treatment means is $\pm 3{\cdot}3$, and from this it is clear that the mean for Treatment I is significantly greater than those for Treatments II and III. The difference between the means for the latter treatments, on the other hand, is quite insignificant. Similarly for the individual transpiration ratios the standard deviation is $\pm 7{\cdot}4$; hence for each time interval the mean for plants of Treatment I is significantly greater than the corresponding means of those receiving higher supplies of phosphorus, but in no case is the difference between the means of these higher treatments sufficiently large to be significant.

DISCUSSION.

It has been shown that an increase in the phosphorus supply from a low to a moderate value had the effect of lowering the transpiration ratio by 10-30 per

cent. according to the time interval over which the ratio was determined. The reduction, however, was not increased by a greater supply of phosphorus. This lack of response is to be expected since with both Treatments II and III the nitrogen supply was limiting at a comparatively early stage in the life-cycle, and in consequence the growth curves were almost identical.

TABLE 1.

Mean Dry Weight Values in grams.

Harvest.	Day.	Treatment. I	II	III
Shoots				
3	19	0·048	0·060	0·060
4	26	0·081	0·153	0·141
	30	*0·11*	*0·28*	*0·24*
5	40	0·234	0·888	0·692
Whole Plant				
	30	*0·20*	*0·36*	*0·30*
5	40	0·43	1·15	0·87
6	65	1·57	9·16	7·68
7	92	6·72	27·17	27·00
8	120	19·41	48·24	48·19
9	153	33·62	68·88	67·82
10	166	36·46	67·41	64·59

The italic figures are derived by interpolation.

The Growth Effect.

The data were next subjected to a graphical analysis similar to that which was carried out by Ballard (1933). Extra values for W were obtained by interpolation on the growth curves (fig. 1), and curves were drawn of $W/(W_{t_2} - W_{t_1})$ for the intervals ending at harvests 6-10. These curves are presented as figs. 2-6. Where W_{t_1} is small in comparison with W_{t_2} (harvests 7-10) the curves approximate to those of W/W_{t_2}, and it will be seen that, at any point prior to harvest, the ratio of the amount of dry matter produced after that point to the amount produced before it is in most cases greater for Treatment I than it is for the other treatments. This is in agreement with the findings of Burgevin and Guyon (1933). It is noteworthy that in this respect phosphorus and nitrogen produce opposite effects.

The effect on transpiration ratio which would be produced by the growth effect, if r were independent of time and treatment, has been assessed from equation (2). Values for the expression

$$\int_{t_1}^{t_2} \frac{W}{W_{t_2} - W_{t_1}} \, dt$$

were obtained for the three treatments by measuring the areas under the curves in figs. 2-6 with a planimeter. These values have been compared with the ob-

served transpiration ratios by adopting Treatment I as a standard (Table 3, *b*). It is obvious (except in the case of harvest 6 which will be discussed later) that the growth factor tends to increase rather than to decrease the ratio with increas-

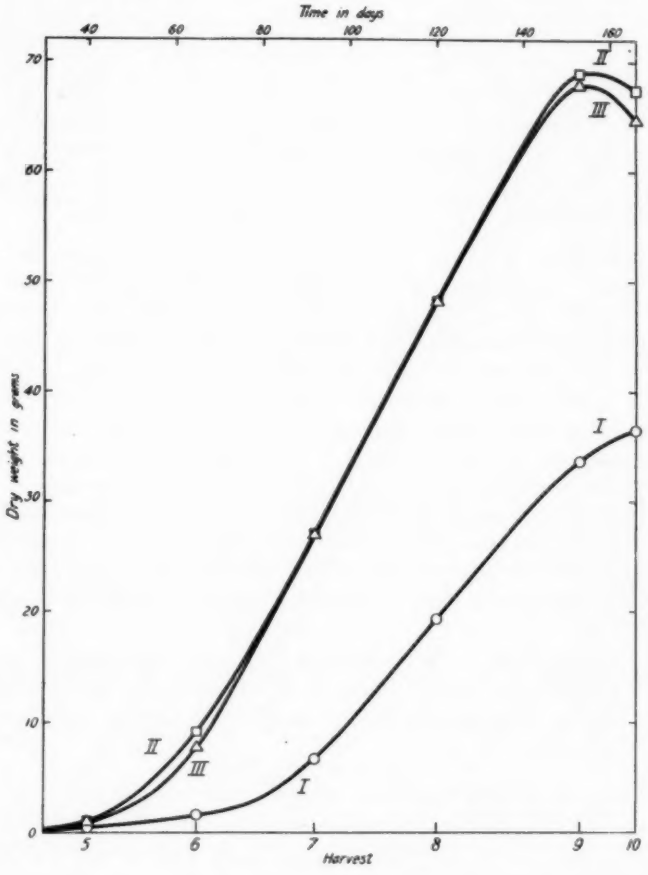

Fig. 1. Growth curves.

ing phosphorus supply. It follows that the transpiration rate, *r*, is the dominant factor producing the observed reduction of transpiration ratio with treatment. It has therefore been considered advisable to evaluate this factor and to investigate its interaction with the growth factor.

TABLE 2.

Mean Transpiration Ratios.

Time Interval	Treatment I	Treatment II	Treatment III
Day 30 to harvest 6	280	199	211
" " " " 7	244	186	187
" " " " 8	246	217	216
" " " " 9	313	282	281
" " " " 10	356	313	312
Treatment mean	287·8	239·3	241·2

Standard deviation of individual values = ±7·4
Standard deviation of treatment means = ±3·3

Transpiration Rate.

The steps taken to obtain values for the transpiration rate per unit dry weight of plant were the same as those used by Ballard, except that all the data for water loss were pooled to produce a single set of curves of cumulative water loss (fig. 7) instead of treating each harvest separately. The curves of 2-daily transpiration per pot (fig. 8) exhibit fluctuations which correspond in time with fluctuations in the evaporating power of the air as measured by two Livingston porous cup atmometers.[1] Points on these curves (particularly maxima and minima) were then selected, and the values of their ordinates divided by interpolated dry weight values appropriate to time and treatment. The quotients thus constitute values of r, and are plotted against time for each treatment in fig. 9. In each case r clearly falls throughout the greater part of the experiment; more important, however, is the fact that after day 67 the values of r for plants of Treatment I remain consistently higher than those for the plants of the other treatments.

Since the growth factor varies so much with time and treatment no attempt will here be made to evaluate the influence of r alone on the transpiration ratios. In the next section, however, the interaction of the two factors will be examined in detail.

The Interaction of the Factors determining Transpiration Ratio.

The drifts in the product of the factors r and $W/(W_{t_2} - W_{t_1})$ for the five time intervals over which the transpiration ratios were determined have been presented in figs. 11-15. From equation (2) the areas under the curves should be proportional to the observed ratios. Accordingly the areas were measured, and the results appear in Table 3, *a*. These figures provide a check on the graphical analysis, and a comparison of the observed and derived values reveals a degree of agreement which is good in view of the assumptions involved in the production of the latter.

[1] Two of these were set up with the experimental plants in the glass-house. It was not considered necessary to record the data in the present paper.

Fig. 2.

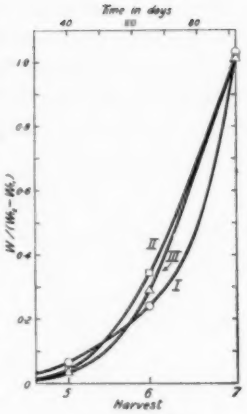

Fig. 3.

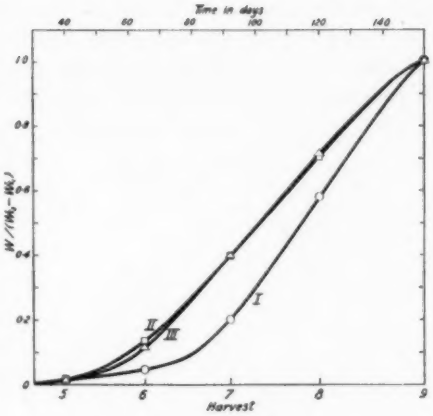

Fig. 5.

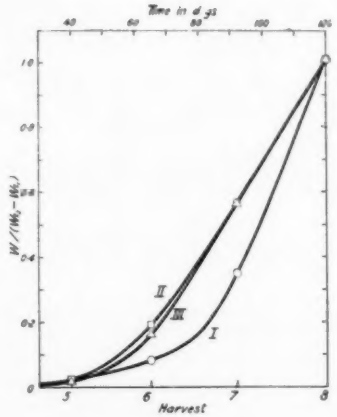

Fig. 4.

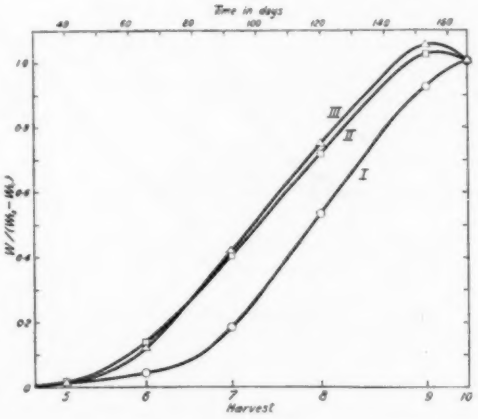

Fig. 6.

Figs. 2–6. Curves showing the drifts in the growth factor, $W/(W_{t_2}-W_{t_1})$, for each treatment over the five time intervals commencing from day 30 and ending at harvests 6–10 respectively.

It has been shown that there are no significant differences between the observed transpiration ratios of the plants of Treatments II and III. Further examination of figs. 11-15 will therefore be confined to a comparison of the curves for Treatments I and II. Fig. 14 (harvest 9) shows that over the interval day 30 to day 100 the product of the two factors is greater for plants of Treatment II than it is for those of Treatment I; the area between the curves over this interval has been designated *A*. Between day 112 and the day of harvest the re-

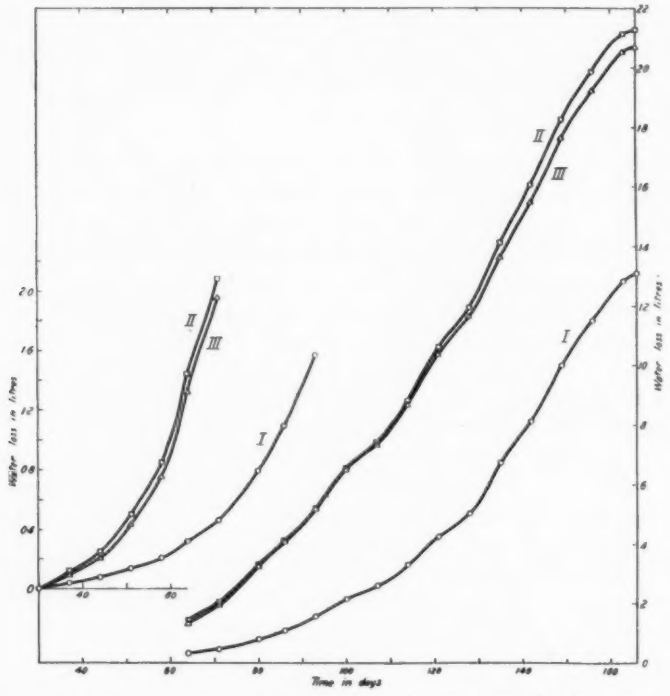

Fig. 7. Curves showing mean cumulative water loss by transpiration. The early portions of the curves have been drawn on an enlarged scale on the left.

lationship is reversed; the area has here been called *B*. Since the whole area under the curve for Treatment II is less than that for Treatment I it follows that *B* is greater than *A*. The reduction in transpiration ratio must therefore be due mainly to a factor or factors operating during the 40 days prior to harvest, and an examination of figs. 5 and 9 shows that *r* is the factor concerned. The curves for the time intervals ending in harvests 7 and 10 show that the general picture is the same in these cases as that described above for harvest 9.

At harvest 8 the derived transpiration ratios for Treatments I and II are

approximately equal and therefore A is approximately equal to B (fig. 13). It is suspected that the mean dry weight per pot for the pots of Treatment I harvested at harvest 8 was higher than that of the remaining Treatment I pots at that time.[2] Since the water loss data are based on the means of all pots not previously harvested, this would tend to lower the transpiration rate before and after the date of harvest. The supposed deviation from the true dry weight value would also diminish the intermediate values of the growth factor for Treatment I (fig. 4). If the surmise is correct it follows that the

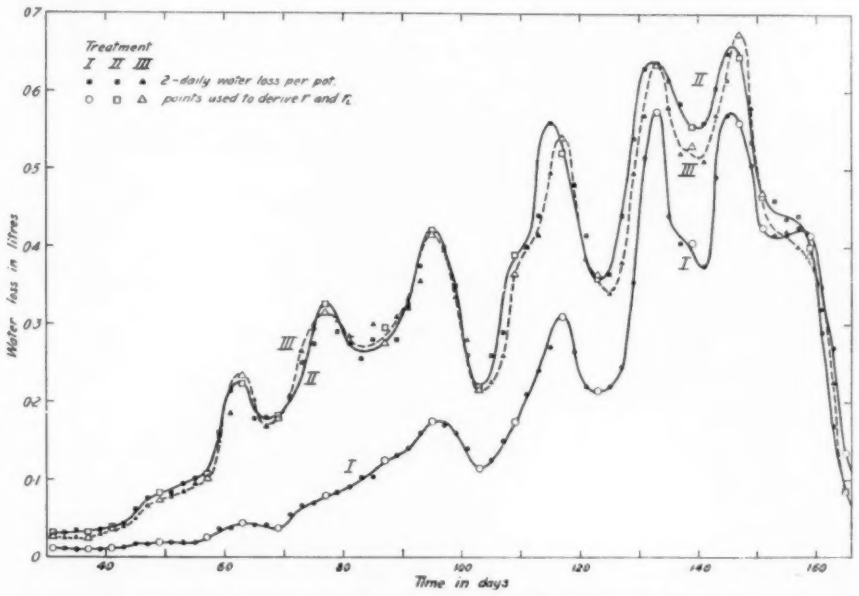

Fig. 8. Curves showing the drifts of mean 2-daily transpiration per pot.

curve for Treatment I in fig. 13 is not as high as it would have been if a better estimate of the mean dry weight had been obtained. In the latter case B would become greater than A, and an explanation would be provided for the discrepancies between the observed and derived ratios for this harvest.

The curves for harvest 6 (fig. 11) have little in common with the others. The product of the factors for Treatment II remains below that for Treatment I throughout, and an examination of figs. 2 and 9 indicates that the growth factor in this case is primarily responsible for the reduction in transpiration ratio.

[2] During the fortnight prior to harvest the plants of these pots lost about 20 per cent. more water per plant than was lost during the same period by the plants of the Treatment I pots harvested at later dates. This effect was possibly due to position in the glass-house.

Except in the early stages of the life-cycle, then, the growth factor tends to cause the transpiration ratio to increase with an increased supply of phosphorus. This tendency is increased by the drift of r with time. In comparison with its values during the final portion of the life-cycle, r has high values during the period when the effect of treatment on the growth factor is high. On this account the effect of treatment produced by the growth factor on the transpiration ratio tends to be weighted. Nevertheless, towards the close of the interval over which the ratio is determined, r more than counterbalances the effect of the growth factor, and brings about the observed reduction in the ratio. The growth factor curves increase rapidly from very low values to approximately unity, and inter-treatment differences are least marked when the values are highest; the effect on

TABLE 3.

Observed and Derived Transpiration Ratios.

Harvest.	Treatment I.		Treatment II.			Treatment III.		
	Observed.	*a*	Observed.	*a*	*b*	Observed.	*a*	*b*
6	280	*254*	199	*171*	190	211	*195*	177
7	244	*229*	186	*197*	301	187	*192*	278
8	246	*220*	217	*222*	342	216	*217*	330
9	313	*338*	282	*285*	405	281	*286*	403
10	356	*373*	313	*321*	477	312	*330*	487

a. Values derived from the graphical determination of $\int_{t_1}^{t_2} \frac{W}{W_{t_2} - W_{t_1}} r dt$.

b. Values derived from the graphical determination of $\int_{t_1}^{t_2} \frac{W}{W_{t_2} - W_{t_1}} dt$, assuming r independent of time and treatment.

transpiration ratio of a given treatment difference in r will therefore be maximal towards the close of the interval. For the intervals ending at harvests 7-10 of the present experiment there is a marked decrease in r with treatment during the final portion of the interval. The dominance in these cases of r as a determinant of transpiration ratio is thus explained.

Further Analysis of Transpiration Rate per Unit of Plant.

It is proposed now to submit the effect of phosphorus supply on r to further analysis. If r_L is the transpiration rate per unit of leaf, and W_L the leaf weight, then

$$r = r_L \frac{W_L}{W}.$$

This equation resolves r into quantities of greater physiological significance, W_L/W being the leaf weight ratio (Inamdar, Singh, and Pande, 1925).

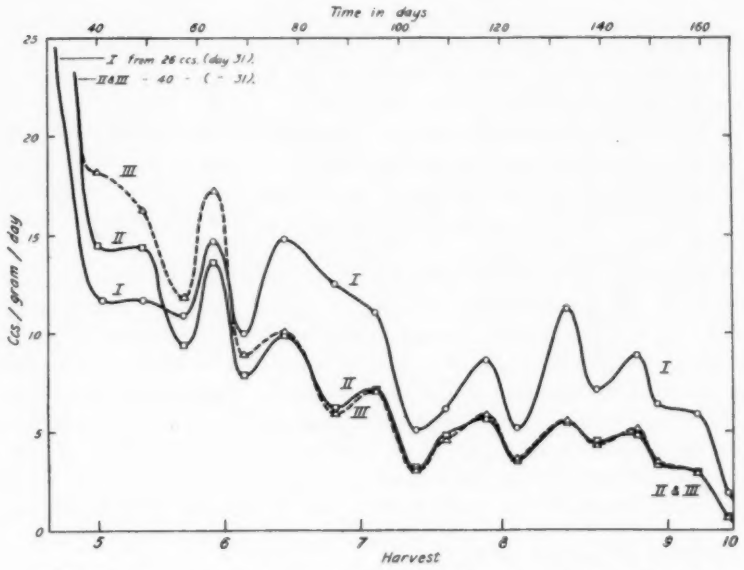

Fig. 9. Transpiration rates per unit dry weight of the whole plant (r).

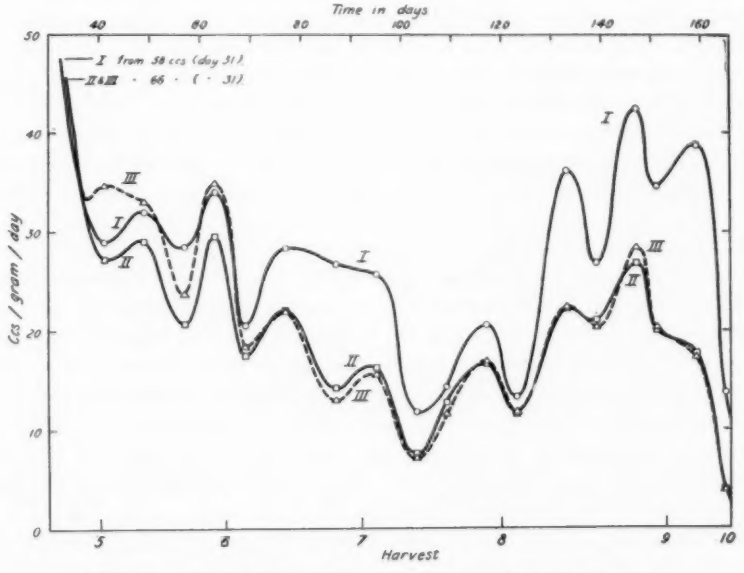

Fig. 10. Transpiration rates per unit dry weight of leaf (r_L).

In the present instance values of r_L were calculated from the 2-daily transpiration rates per pot and interpolations on smoothed curves of W_L; they are plotted against time for each treatment in fig. 10. The values of leaf weight ratio are given in Table 4.

It is clear that the drift of r is influenced by the characteristic fall in W_L/W during maturity and senescence, although it is more markedly influenced by the general drift and fluctuations of r_L (cf. figs. 9 and 10). Ballard attributed the fall with time in r solely to the fall in W_L/W, but a fall in r_L could also have been a contributing factor.

With regard to treatment effect on r, at harvest 5, and to a less extent at harvest 6, W_L/W is increased by treatment, whereas the effect on r_L is comparatively small; it follows that treatment differences in r at this time are mainly determined by the differences in W_L/W. In the region of harvest 8, on the contrary, W_L/W is markedly depressed by treatment. This contributes to the comparatively high values of r for Treatment I, although the consistently high values of r for this treatment after day 67 are caused mainly by the effect of treatment on r_L. Ballard found no significant effect of nitrogen supply on r_L, so that all effect of treatment on r was attributable to its effect on the leaf weight ratio.

Transpiration Rate per Unit of Leaf.

It was considered of interest finally to calculate the relative transpiration (Livingston, 1906), which is the ratio of the rate of transpiration to the rate of evaporation from a free water surface or from the evaporating surface of an atmometer. This quantity is considered as an approximation to a measure of r_L in a constant environment. While it is usually expressed on an area basis, in the present case the values were obtained by dividing the values of r_L by the mean values of water loss from the two atmometers set up with the experimental plants. The values are presented in fig. 16; as was expected they show large fluctuations, but the smoothed curves for the three treatments reveal the presence of a definite ontogenetic drift. In all cases the relative transpiration rate falls rapidly during the early portion of the life-cycle (day 30-100), then remains approximately constant for a period (day 100-150), and finally undergoes a senescent fall. Throughout the experiment r_L has been calculated on the assumption that transpiration occurs only from the leaves. Water loss from the leaf-sheaths may be considered as negligible, but that from the inflorescences, which organs began to appear on day 132, was probably sufficient to delay the senescent fall in the relative transpiration rate. Although successive points on these curves are not strictly comparable, it is highly improbable that the observed drifts are merely an expression of the differential effects of the environment on transpiration and evaporation from the atmometers.

A comparison of fig. 16 with figs. 9 and 10 makes it clear that the early fall in the relative transpiration rate largely determines the general downward

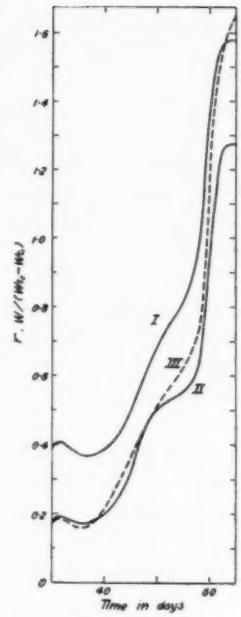

Fig. 11.

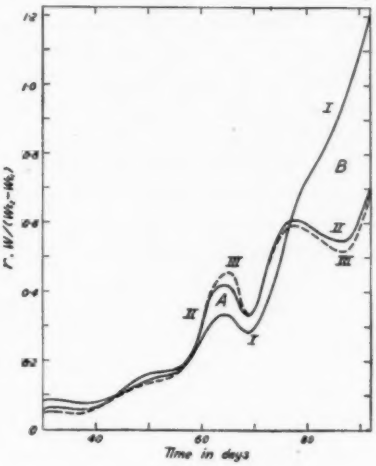

Fig. 12.

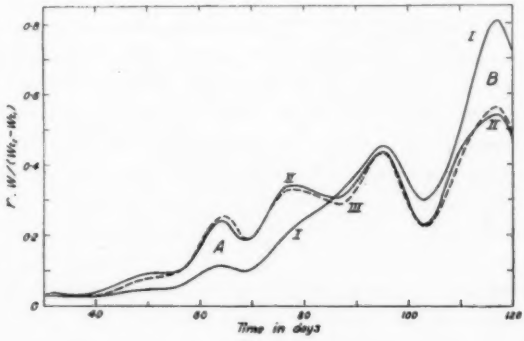

Fig. 13.

Figs. 11–13. Curves showing the drifts in the products of the factors $W/(W_{t_2}-W_{t_1})$ and r for each treatment over the three time intervals commencing from day 30 and ending at harvests 6–8 respectively.

trend of r_L, and also that of r, since the leaf weight ratio is relatively constant at this time. Where the relative transpiration rate is constant, however, variations in r_L are attributable to the environment alone. The inter-treatment relations of the curves of fig. 16 are of course the same as those of the curves of r_L.

It is also apparent from the data that the environment is a factor influencing treatment effects on transpiration ratio. In the present experiment the plants were grown in an environment in which the evaporating power of the air underwent a general increase with time. This partially counteracted the downward trend of r and made its values higher just prior to harvest than would have been the case in a constant environment. Both these influences tend to produce a reduction in transpiration ratio with increased phosphorus supply. Therefore, in the case of a summer annual, if treatment effects on r and the growth factor were as in the present experiment, the reduction in transpiration ratio would be diminished, or even changed to an increase, with increased supply of phosphorus.

Nitrogen Content and Relative Transpiration Rate.

It was observed that, at most harvests, treatment effects on r_L, on interpolated values of relative transpiration rate, and on nitrogen content of the leaves, were in the same direction. The correlation coefficient between the relative transpiration rate and nitrogen content of the leaves was therefore determined. In order to eliminate the drifts of the two variables with time, a regression equation of the form

$$Y = \bar{y} + b_1(x - \bar{x}) + b_2(x^2 - \overline{x^2}),$$

where y is the variable and x time, was fitted to the values for nitrogen content and relative transpiration rate of Table 4; this equation was found to represent adequately the drift in each case. The residuals were then correlated and a coefficient of correlation of $+0{\cdot}64$ was obtained. This coefficient is significant at the 1 per cent. point ($n = 14$) (Fisher). The correlation was determined with the relative transpiration rate and not with r_L, because environmental effects on nitrogen content may be expected to be small in comparison with those on r_L, and also because the elimination of the time drift from r_L would have been impossible.

A similar partial correlation between relative transpiration rate and the water content of the leaves is suggested by the data (Table 4), but the coefficient has not been determined in this case.

An increased nitrogen content is generally accompanied by an increase in water content, and if an increase in water content so produced causes an increase in relative transpiration rate, this may underlie the correlation coefficient above determined. But, in the absence of further knowledge of the system involved, it is not proposed to discuss further the physiological basis of the correlation, nor whether the ontogenetic drift in relative transpiration rate is determined by that of the nitrogen content.

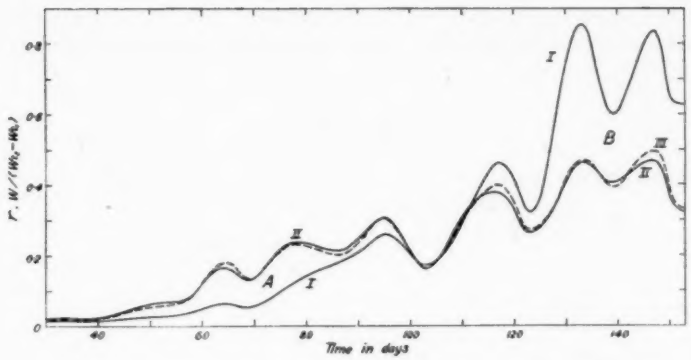

Fig. 14.

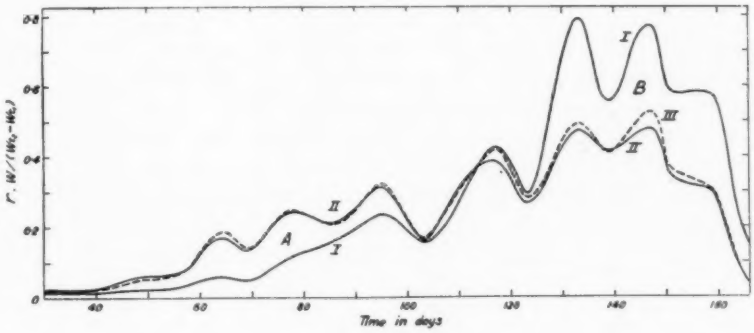

Fig. 15.

Figs. 14–15. Curves showing the drifts in the products of the factors $W/(W_{t_2}-W_{t_1})$ and r for each treatment over the two intervals commencing from day 30 and ending at harvests 9 and 10 respectively.

It would be of interest to know whether there is a similar correlation when the nitrogen content is increased by nitrogen supply. As already stated, Ballard found no significant effect, but it is suggested that further analysis of his data might have revealed an increase in transpiration rate with treatment during some portions of the life-cycle (see particularly harvest 3, Table 6, p. 175 of his paper).

TABLE 4.

Leaf Weight Ratio, Water and Nitrogen per cent. Dry Weight of Leaves, and Relative Transpiration Rate.

	Treatment		
Harvest	I	II	III
Leaf Weight Ratio			
5	0·408	0·533	0·550
6	0·447	0·464	0·498
7	0·443	0·440	0·457
8	0·404	0·321	0·327
9	0·176	0·167	0·168
10	0·133	0·165	0·173
Water Content of Leaves			
5	583	716	670
6	598	615	658
7	458	356	358
8	258	231	224
9	111	55	58
10	64	11	14
Nitrogen Content of Leaves			
5	5·72	6·12	5·99
6	5·78	4·01	4·50
7	4·13	1·40	1·47
8	1·65	0·941	0·917
9	0·991	0·405	0·425
10	0·525	0·328	0·354
Relative Transpiration Rate (Interpolated values)			
5	3·70	3·58	4·09
6	2·78	2·25	2·49
7	1·39	0·85	0·82
8	0·88	0·71	0·70
9	0·93	0·52	0·52
10	0·78	0·22	0·22

Pearsall and Ewing (1929) quote Miss Ryan as having found that in the case of ivy seedlings transpiration rate when measured on a leaf area basis is diminished with increased nitrogen supply. Gregory and Richards (1929) found, however, that the ratio of leaf area to dry weight is increased with increase in the nitrogen supply, so that the above reduction in transpiration rate would probably be diminished and possibly converted into an increase with treatment when placed on a dry weight basis. Gassner and Goeze (1934), using cut leaves

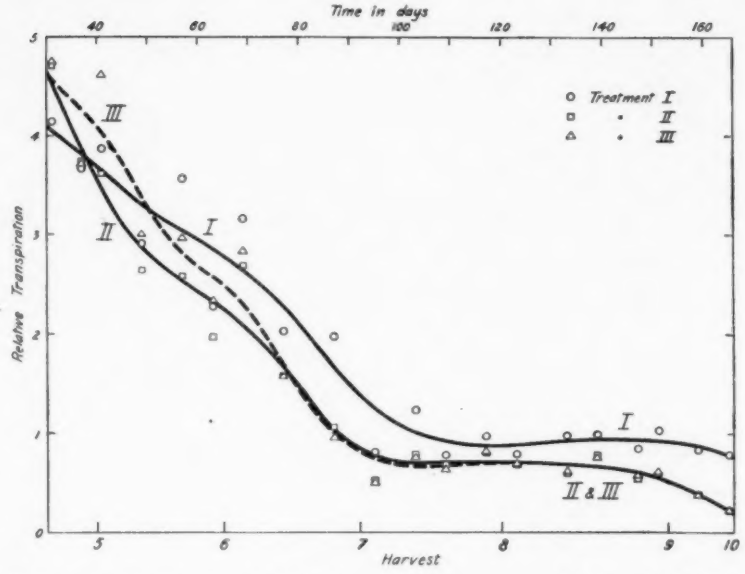

Fig. 16. Smoothed curves of relative transpiration rate per unit dry weight of leaf.

with their petioles in water, found that, provided potassium supply had not been deficient, transpiration rate on a leaf area basis was increased with increasing nitrogen supply. Crist (1926), on the other hand, found that the rate of drying of detached leaves of high nitrogen plants was less than those of low nitrogen plants. One cannot, however, argue from the rate of water loss of detached leaves to that of leaves attached to the plant; the latter is clearly a much more complicated system.

The existing data on this question are thus inconclusive.

ACKNOWLEDGMENTS.

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SUMMARY.

Transpiration ratios were determined for a winter annual (oats) over five different time intervals all commencing from the same early stage in the life-cycle. An increase in phosphorus supply from a low to a moderate amount had the effect of lowering the ratio for each time interval. An additional supply of phosphorus produced no further effect.

Except in the case of the shortest time interval the reduction in the ratio is shown to be due to a decrease with treatment in the transpiration rate per unit weight of plant consequent on (*a*) a decrease with treatment in the transpiration rate per unit weight of leaf and (*b*) a decrease with treatment in the leaf weight ratio during part of the life-cycle.

Increased phosphorus supply also affects transpiration ratio by means of an effect on growth. This effect, except in the case of the shortest time interval, is such that, at any point prior to that of harvest, the ratio of the amount of dry matter produced after that point to the amount produced before it is, in most cases, less the greater the supply: this tends to increase the transpiration ratio; but its effect is more than counterbalanced by the effect of the decrease in the rate per unit of plant. The interaction of the two factors is examined in detail, and the dominance of the rate factor is shown to be due to its effect during the final portion of the interval, at which time the high values of the growth factor increase the effect of the observed treatment differences in the rates. The environment is shown to affect the interaction and hence to influence the effect of treatment on transpiration ratio.

For the shortest time interval the effect on growth is the reverse of that above described, and the reduction in transpiration ratio with treatment is due to the growth factor rather than to any effect of treatment on transpiration rate.

A significant correlation was found between the relative transpiration rate per unit weight of leaf and the nitrogen content of the leaves, and both these quantities underwent a similar decline throughout the greater part of the growing period.

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